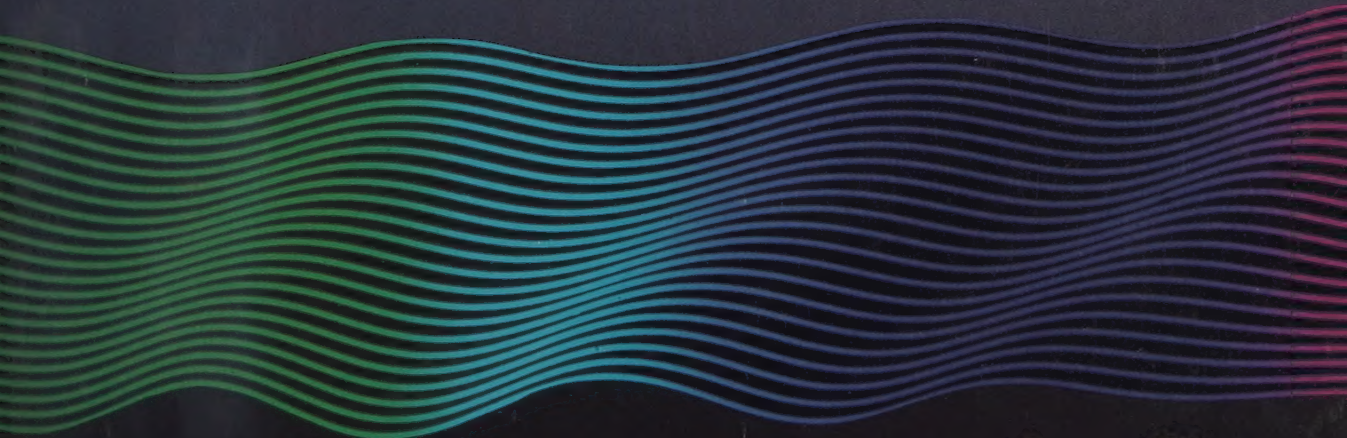




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Demographic Consequences of Age-Specific Dispersal in Marine Fish Populations¹

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The fundamental model of marine fish population dynamics assumes that major events occur within the census area and that immigrants and emigrants can be safely ignored in the evaluation of stock dynamics. Dispersal is generally assumed to be associated with the movement of immature individuals away from their home area. Such a process may produce vagrants that have a low probability of surviving or successfully reproducing elsewhere. An alternative view, developed here, proposes that dispersal can have profound demographic consequences not only for the resident stock but for neighbouring stocks. A review of the fisheries literature and an analysis of data for two Scotian Shelf haddock stocks provided support for the hypothesis that the spatial magnitude of postlarval dispersal is generally density dependent, that vagrants flourish as juveniles outside the home area in neighbouring stocks, that they subsequently return home when mature, and that the recruitment dynamics of the neighbouring stocks are seriously misinterpreted when such movements are ignored. Additional implications of these findings, including synchronous recruitment patterns commonly observed between stocks, density-dependent growth, and groundfish stock management practices, are discussed.

Le modèle de base de la dynamique des populations de poissons marins suppose que des événements importants surviennent dans la zone occupée et que l'on peut ignorer sans danger les apports et retraits, par entrée et sortie, sans que cela ait des répercussions importantes pour l'évaluation de la dynamique des stocks. La dispersion est généralement supposée liée au déplacement des individus immatures qui s'éloignent de leur zone d'origine. Un tel processus peut donner lieu à la production d'individus errants dont la probabilité de survie ou de reproduction est faible. Selon une autre hypothèse, élaborée dans l'article, la dispersion peut avoir d'importantes conséquences démographiques tant pour le stock d'origine que pour les stocks voisins. Un examen des publications sur les pêches et l'analyse de données relatives à deux stocks d'aiglefin du plateau néo-écossais appuient l'hypothèse selon laquelle l'importance spatiale de la dispersion post-larvaire est généralement dépendante de la densité, les individus errants se développent en juvéniles au sein des stocks voisins avant de revenir dans leur zone d'origine au moment où ils deviennent matures et la dynamique du recrutement des stocks voisins est interprétée de façon fortement erronée lorsqu'on ne tient pas compte de ces déplacements. D'autres incidences, notamment les régimes de recrutement synchrones couramment observés entre stocks, la croissance en fonction de la densité et les pratiques de gestion des stocks, sont aussi traitées.

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Population dynamics models of exploited fish stocks generally ignore dispersive processes such as immigration and emigration (e.g. Sissenwine 1981). It is typically assumed that major population events occur within the census area. This is equivalent to stating that the management unit represents a discrete population. While acknowledged to be a dubious assumption for Atlantic herring (*Clupea harengus*) in the north-west Atlantic (Sinclair et al. 1985), this is judged quite acceptable for commercial groundfish stocks, such as Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*), in this region. Halliday and Pinhorn (1990) have noted that "in the history of management through TACs (total allowable catches) of northwest Atlantic groundfish, which now extends for close to 20 years, stock boundaries have not often arisen as the potential basis for scientific or management problems." Consequently, time series estimates of abundance, reconstructed from commercial landings and fishery independent trawl surveys from a given management unit, are generally con-

sidered to be adequate descriptors of groundfish stock dynamics.

It is perhaps logical to consider emigrants as losses to the population and therefore demographically unimportant. A widely accepted early viewpoint promoted by population ecologists was that dispersal acted as a "safety valve" providing immediate relief to overpopulation (Wynne-Edwards 1962). Individuals would leave home (vagrants) when conditions were intolerable in favour of a very low probability of surviving long enough to reproduce elsewhere. Although this viewpoint has been greatly modified by terrestrial ecologists (see Lidicker 1975, 1985), its original elements have reappeared in recent thinking about marine fish populations (i.e. the member/vagrant hypothesis; Sinclair 1988). Sinclair uses the term "vagrancy" to refer to spatial losses of eggs and larvae due directly to advection and diffusion, failure of juveniles to recruit to their parent population (juvenile vagrants), and wandering of adults (adult vagrants).

However, some fish stocks are known to be strongly coupled by dispersive processes. The best documented example is that of the cod stocks of Iceland and West Greenland which are

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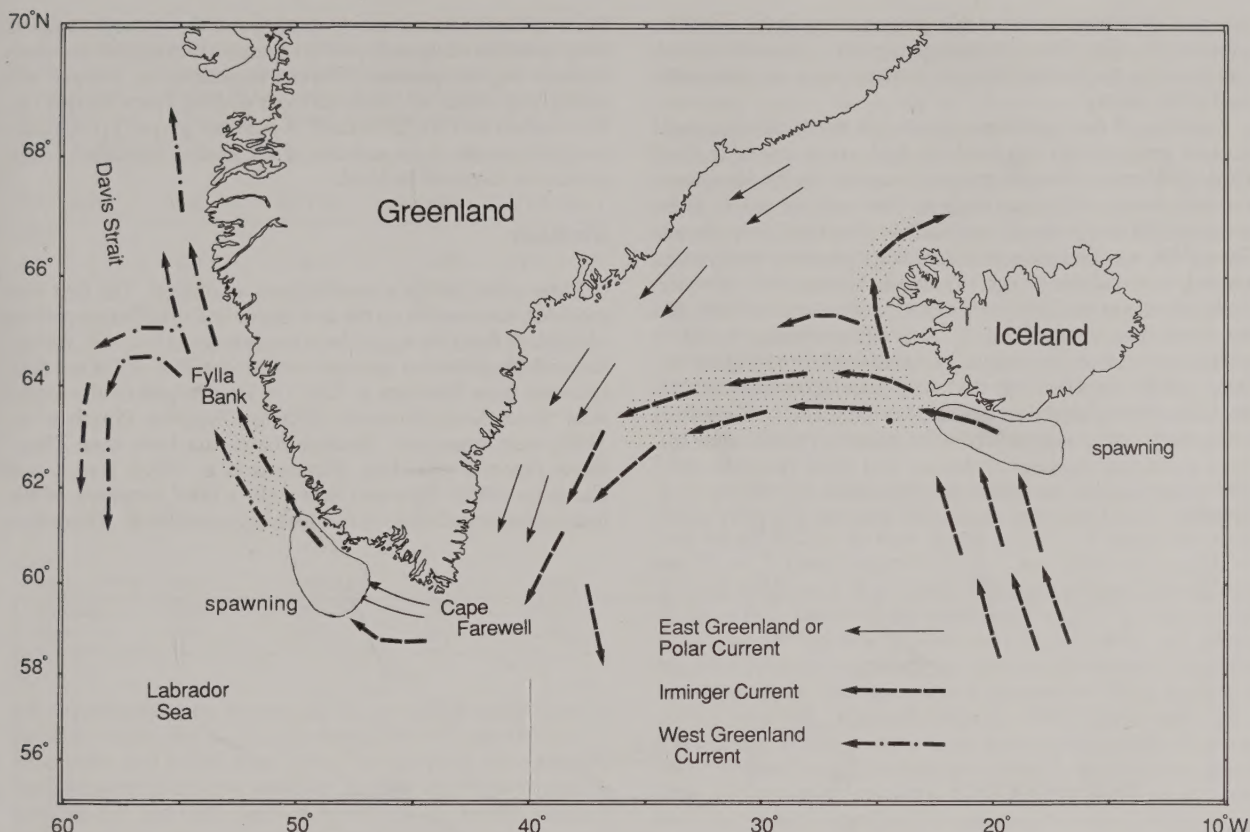


FIG. 1. General circulation and distribution of postlarval cod (shown by stippling) in Iceland and West Greenland waters based on descriptions by Horsted (1989) and Hovgård and Buch (1990). Some year-classes inhabiting the waters off West Greenland are a mixture of resident and Icelandic cod, a situation arising when strong year-classes are produced at Iceland and/or when the velocity of the Irminger Current is anomalously high. Emigrant cod return to Icelandic waters for spawning.

TABLE 1. Characteristics of the dispersive processes influencing cod population dynamics at Iceland.

A. Emigration

1. Dispersers are juveniles (or late-stage larvae)
2. Strong year-classes more prone to disperse (i.e. density dependent), but exceptions exist
3. A physical oceanographic component (Irminger Current) contributes to unidirectional transport of the 0-group
4. Migrants contribute to a stock which flourishes periodically (at West Greenland)

B. Return migration

1. Timing is linked to maturation
2. Magnitude of return (or fidelity to home site) may depend on what life stage was dispersed
3. The situation is detectable using age-structured fisheries data such as surveys and commercial landings

separated by a distance of about 2000 km. Tagging studies, research surveys, and commercial fisheries data have shown that in some years, 0-group cod originating from Iceland mix with the resident cod stock at West Greenland (see reviews in Horsted 1989; Hovgård and Buch 1990; Schopka 1991). After a residence time ranging from 5 to 7 yr the emigrants undertake a 2- to 3-yr return migration to the spawning grounds off the southwest coast of Iceland. This leads to periodic requirements

for upward revisions of the initial estimates of year-class strength for the Icelandic cod stock. Not all year-classes, nor all individuals in a given year-class, emigrate from Iceland. Rather, members of strong year-classes produced in Iceland have a greater tendency to invade West Greenland waters. Variability in the velocity of the Irminger Current, which averages 20 cm/s, also appears to play an important role in transporting postlarval cod from Iceland to Greenland (Fig. 1). This current has been implicated in the periodic or ephemeral character of the West Greenland cod stock which, in the last two centuries, has experienced three boom periods and one bust that lasted nearly 70 yr (Buch and Hansen 1986; Hovgård and Buch 1990). Hence, the annual assessment of the West Greenland cod stock incorporates an age-specific emigration coefficient (E), in addition to natural mortality (M) and fishing mortality (F), in the estimation of total mortality ($Z = M + F + E$). E is negative and variable for ages 3–5 and is interpreted as additional recruitment to the resident stock at West Greenland. E is positive for ages >6 yr and averages about 25% per year, reflecting the return movement of Icelandic fishes to their home waters (Jones 1978; Anonymous 1987; Schopka 1991). Table 1 presents a summary of the salient features of this two-cod-stock inter-relationship from the Icelandic cod stock perspective.

Unless these northern cod stocks are a biological oddity, it is unwise to reject the notion that dispersive processes have no demographic consequences for marine fish populations. Rather,

we must determine whether this dispersive model is valid for other stock pairs. This can be achieved by systematic examination of the spatial dynamics of year-classes at various stages in the life history.

A review of the literature suggests that the dispersive model may be applicable to two haddock stocks on the Scotian Shelf (Fig. 2) where a dramatic reversal occurred in the abundance estimate for the 1983 year-class for both resident stocks in the area (NAFO Divisions 4X and 4VW). The 1983 year-class in Div. 4VW was first judged to have been relatively weak based on its low abundance at age 1 (juveniles) in research trawl surveys (Mahon et al. 1985). However, subsequent to 1988, this year-class was estimated to be well above average based on abundances indices for this year-class at ages 4 and 5 (Zwanenburg 1989). In Div. 4X the 1983 year-class was initially declared to be a failure on the basis of extremely low numbers of haddock larvae encountered during monthly broad-scale surveys conducted between February and June (Koslow et al. 1985). Subsequent analysis of this year-class by O'Boyle et al. (1989) revealed the 1983 year-class to be only slightly below

the long-term mean level. Assuming that the surveys sampled the population adequately, these apparent reversals in year-class strength beg the question: how can something (an average and strong year-class) be produced from nothing (very limited larval numbers and few juveniles)? A possible answer to this question may reside in an analysis of the spatial dynamics of the postlarval stages of haddock.

Methods

Three principal data sources were examined. The first two provided information on the abundance and distribution pattern of haddock from the egg to the juvenile stage in Div. 4X. Broad-scale ichthyoplankton surveys conducted in Div. 4X at monthly intervals from February to June 1983–85 as part of the south-west Nova Scotia Fisheries Ecology Program (Smith et al. 1989) were examined. Because these data have been extensively reported elsewhere (Campana et al. 1989; Hurley and Campana 1989), I present here only a brief summary of the interannual abundances of haddock eggs and larvae. These data

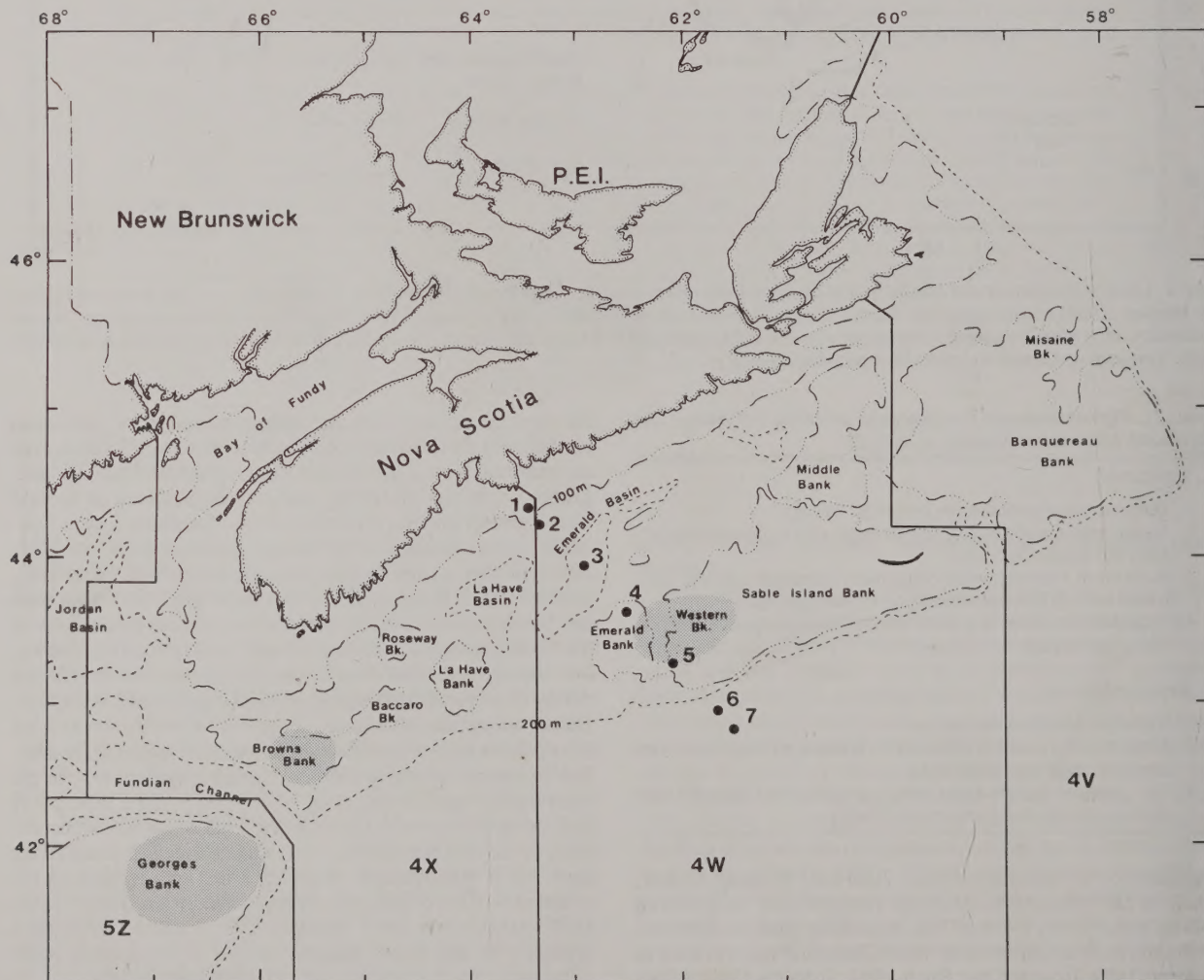


FIG. 2. Map of the Scotian Shelf showing locations of offshore banks supporting spawning populations of haddock in NAFO Div. 5Z, 4X, and 4VW. Numbers 1–7 indicate station locations along a standard hydrographic section running southeast from Halifax. Stippling shows haddock spawning locations.

TABLE 2. Evolution of three haddock year-classes in NAFO Div. 4X based on stage-specific surveys of abundance: 1, number per square metre except for juveniles (number per standard tow); 2, frequency of nonzero sets; 3, number of sets. Also shown is estimate of year-class strength at age 1 based on a virtual population analysis (VPA) by O'Boyle et al. (1989).

Year-class	Egg	Larvae	0-Group juveniles	VPA age 1
1983				
1	0.36	0.01	9.68	27.3×10^6
2	0.29	0.04	0.67	—
3	157	157	54	—
1984				
1	1.88	0.86	0.60	17.5×10^6
2	0.53	0.26	0.37	—
3	169	169	48	—
1985				
1	4.04	1.04	0.36	4.3×10^6
2	0.66	0.54	0.14	—
3	179	179	50	—
Rank				
High	1985	1985	1983	1983
Medium	1984	1984	1984	1984
Low	1983	1983	1985	1985

are supplemented by surveys of the pelagic juvenile stage of haddock conducted in Div. 4X during 20–30 June 1983, 4–11 June 1984, and 17–27 June 1985. The results of these juvenile surveys have also been reported: Koeller et al. (1986) examined the day and night depth distributions of juvenile haddock and their horizontal distribution from the June 1983 survey, and Campana et al. (1989) calculated absolute abundance estimates of juvenile haddock from a subset of the total data available for construction of survival curves in 1983–85. Methodological details of the juvenile surveys are provided by these authors. No comparable survey exists for the Div. 4VW haddock stock.

The third data source was research vessel (RV) surveys conducted each year in July since 1970 in both Div. 4X and 4VW. Both sampling and data analysis protocols are well documented (see Doubleday 1981; Halliday and Koeller 1981; Smith and Somerton 1981). The RV surveys provide reliable indices of abundance and distribution by age for the groundfish stocks on the Scotian Shelf.

Results

Haddock egg abundance in Div. 4X, averaged over the April, May, and June surveys, was lowest in 1983 and highest in 1985 (Table 2). Larval abundance was also lowest in 1983, with the margin of difference exceeding two orders of magnitude relative to 1985. Based on abundance at the larval stage, the outlook for the 1983 year-class was bleak compared with 1984 and 1985 (Table 2). Only nine haddock larvae were collected in six monthly surveys in 1983, leading Koslow et al. (1985) to predict a year-class failure for Div. 4X haddock.

Except for 1983 when too few larvae prohibited meaningful centroid estimation, centroids of the horizontal distributions of haddock eggs and larvae from the monthly surveys in Div. 4X were invariably located in the vicinity of Browns Bank, the principal spawning location for haddock in Div. 4X (O'Boyle et al. 1984). Peak egg and larval concentrations occurred on the Bank within the 100-m isobath (Hurley and Campana 1989).

Juvenile haddock (0-group) in Div. 4X were very abundant in the 1983 survey compared with both 1984 and 1985. Nearly 70% of the 54 sets yielded haddock (Table 2), and several sets produced catches in excess of 25 per tow (Fig. 3). No sets exceeded 25 haddock per tow in the other two years. What is remarkable about the high abundance of juveniles in 1983 is that abundance at the larval stage was low relative to 1984 and 1985 (Table 2). The 1983 haddock juvenile survey in Div. 4X also featured the highest abundances and the greatest frequency of nonzero sets along the eastern boundary of the survey (Fig. 3). This is reflected in the northeasterly position of the centroid relative to the principal spawning location for haddock in Div. 4X, Browns Bank, and in the high along-shelf variance in the horizontal distribution of juveniles. The centroids of the horizontal distribution of juvenile haddock in 1984 and 1985 were in the vicinity of Browns Bank which is distinctly different from the easterly bias characteristic of the 1983 year-class (Fig. 3).

In addition to being more abundant in 1983, juvenile haddock in Div. 4X were, on average, larger as well (1983: average total length (TL) = 4.4 cm, range = 2.1–7.9 cm; 1984: average TL = 3.2 cm, range = 2.2–4.1 cm; 1985: average TL = 3.4 cm, range = 2.5–4.6 cm). The approximate average age of these fish, based on the posthatch to 200-d growth model developed for haddock by Bolz and Lough (1988), and allowing 14 d for hatching using the relationship between haddock egg development rate and ambient temperature (Page and Frank 1989), was 100, 85, and 90 d for 1983, 1984, and 1985, respectively. There was also a pronounced size gradient in June 1983 with mean total length progressively increasing from about 4 cm at the eastern boundary of the survey area to about 6 cm near the western boundary, a distance of nearly 200 km ($r = 0.73$, $n = 8$, $p < 0.05$). No such gradient was evident in 1984 or 1985.

Collectively, this information suggests a significant input of juveniles into the Div. 4X stock area in June 1983. Both the reversal in ranking from the larval to juvenile stage (Table 2) and the east to west gradient in size suggest that the juveniles sampled in June 1983 were immigrants from other stocks located to the east of Browns Bank. It is noteworthy that at no time did a reversal in ranking at the egg or larval stage occur (Table 2). In this respect the haddock on the Scotian Shelf conform to the dispersal model, i.e. the dispersers are juveniles (Table 1). The similarity of ranks between the juvenile stage and abundance at age 1, based on a virtual population analysis of Div. 4X haddock (O'Boyle et al. 1989), also suggests that these fish remained in the Div. 4X stock area for a considerable time after their invasion as 0-group.

The most likely source of these immigrants is the eastern Scotian Shelf (Div. 4VW) haddock stock. Peak spawning in the Div. 4VW stock occurs at approximately the same time as spawning in Div. 4X (Page and Frank 1989). Div. 4VW haddock spawning is concentrated on Emerald Bank and Western Bank in Div. 4W (O'Boyle et al. 1984), slightly more than 300 km to the east of Browns Bank. There is no evidence that haddock spawn inshore in either Div. 4X or 4VW (O'Boyle et al. 1984; Hurley and Campana 1989).

The gross features of the circulation on the Scotian Shelf were determined from mean monthly geostrophic volume transport calculations at 10-m depth intervals from the surface to 50-m between stations 4 and 5 along the Halifax section (Fig. 2). Estimates of interannual variability of geostrophic transports along the Halifax section are not reliable, given the available sampling frequency (Drinkwater et al. 1979). The

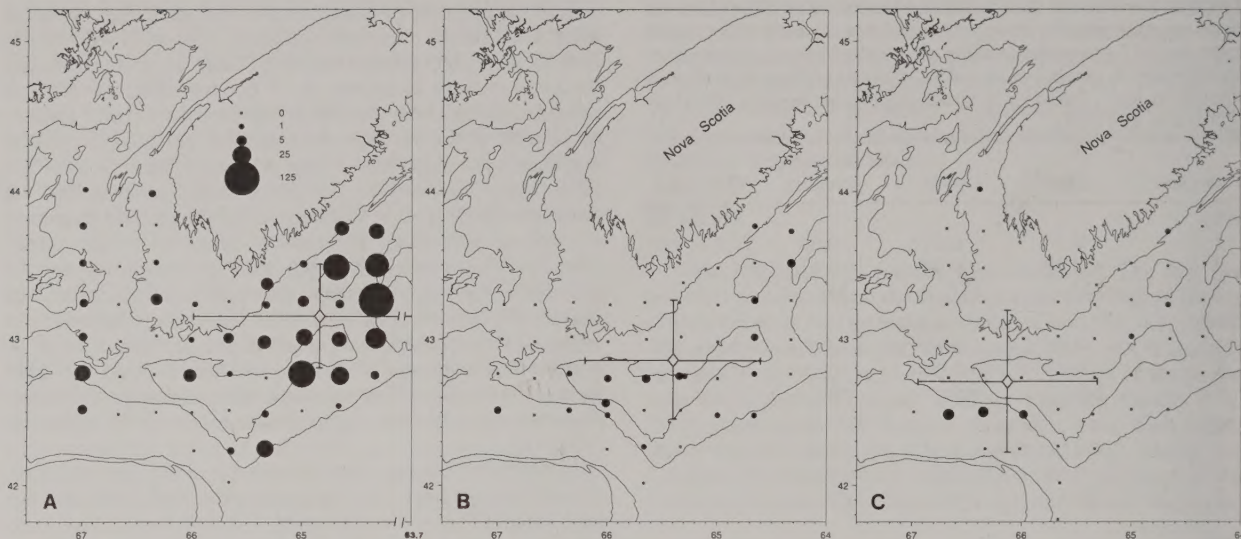


FIG. 3. Expanding symbol plot of juvenile haddock catch per tow from International Young Gadoid Pelagic trawl surveys conducted in June of (A) 1983, (B) 1984, and (C) 1985. Centroid positions and second moments of the juvenile distributions are also shown.

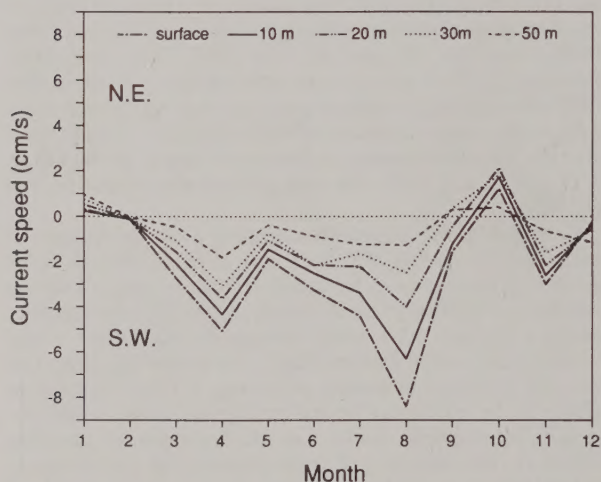


FIG. 4. Geostrophic currents estimated between stations 4 and 5 along the Halifax section. Data used in the geostrophic calculations were for the period 1952–71. See Drinkwater et al. (1979) for details on analytical methods and assumptions.

upper 50 m is considered the relevant depth zone for the transport of the pelagic life stages of haddock, given that Frank et al. (1989) found egg and larval stages to occur in the upper 40 m of the water column and Koeller et al. (1986) established that juvenile haddock are found near the surface during both day and night, with the highest concentrations occurring at depths <60 m. The results show that during the pelagic phase of haddock (April–July), water transport is from the northeast to southwest at all depths examined. The current velocity, which averages about 2–3 cm/s, decreases with depth (Fig. 4). The magnitude of this southwestward subsurface current is consistent with the mean age of the juveniles (100 d) encountered during the June 1983 survey and the distance (250 km) separating the centroid position of juveniles in Div. 4X and the Western Bank haddock spawning location in Div. 4W. Georges Bank,

located 100 km to the west (Fig. 2), also supports a spawning stock of haddock. However, the Fundian Channel and the strong recirculation feature on Georges Bank (Loder et al. 1988) serve to keep its haddock stock relatively isolated (Clark and Vladikov 1960; also see review in Bowen 1987). Georges Bank is not therefore a viable source of immigrants.

The dispersal model predicts that emigration from Div. 4VW, and hence juvenile immigration to Div. 4X, is dependent on year-class strength (Table 1). This leads to the expectation that the 1983 haddock year-class in Div. 4VW, which I hypothesize contributed to the strong 1983 juvenile abundance in Div. 4X, should be above average. As noted earlier, this year-class was initially estimated to be weak but was later revised upward. Thus, relative to the 1968–85 average recruitment of approximately 15×10^6 haddock at age 2, the 1983 year-class estimate of 22.85×10^6 individuals was nearly 52% higher (Zwanenburg 1989). The fact that the upstream haddock stock produced a strong year-class in 1983 is therefore consistent with the hypothesis proposed.

I examined data from RV surveys of these haddock stocks to further evaluate the 1983 year-classes in Divs. 4X and 4VW and to determine whether haddock year-classes on the Scotian Shelf other than those produced during 1983–85 were subject to similar major revisions.

Assuming that the 1983 haddock year-class in Div. 4X originated from immigration from the upstream Div. 4VW stock as hypothesized, a return migration at the age of maturity would be expected (Table 1). Age at maturity should determine the duration of mixing in the downstream stock. Waiwood and Buzeta (1989) established that for haddock in southwestern Nova Scotia, both males and females typically reach 50% maturity at lengths between 37 and 43 cm (ages 3–3.5). Therefore, for each haddock stock, I compared the cumulative abundance of each year-class when its members were immature (i.e. the summation of annual abundance indices at ages 1–3 from the RV surveys) relative to when its members were mature (ages 4–6). Because several years are required to generate the relevant data for a given year-class, 1985 was the last year-class

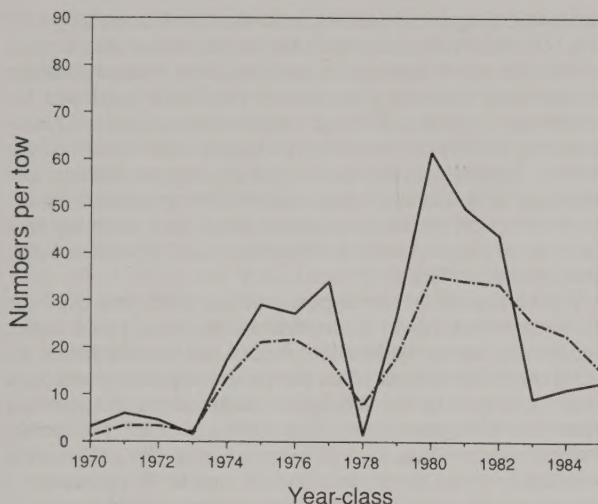


FIG. 5. Cumulative index of abundance of a year-class when it is immature (ages 1–3) and mature (ages 4–6) for the 1970–85 year-classes of Div. 4VW haddock. Analysis is based on data from annual research vessel trawl surveys on the Scotian Shelf conducted in July since 1970. Data taken from Zwanenburg (1992). The solid and broken lines show immature and mature indices, respectively.

that could be analyzed in this manner. Age 0 data from the surveys was excluded because variable timing of the transition from pelagic to demersal phases from year to year may bias the estimates. Templeman et al. (1978) and Templeman and Bishop (1979) have analyzed survey data in a similar manner for haddock stocks on the southern Grand Bank and St. Pierre Bank.

The 1983 haddock year-class in Div. 4VW was nearly threefold more abundant when mature than when immature (Fig. 5). This is consistent with the hypothesis that the 1983 year-class in Div. 4VW, a proportion of which dispersed to Div. 4X from their home site as juveniles, returned home when mature. This would explain the need for an upward revision of this year-class based on survey abundance indices of mature fish.

Other year-classes, in particular that spawned in 1978, were also more abundant in the Div. 4VW census area when mature (Fig. 5). Mahon et al. (1985) gave special consideration to the 1978 year-class in the Div. 4VW haddock assessment. These authors observed "At ages 1–3 this yearclass appeared to be very poor in both the survey and commercial catch. In 1983 and 1984 its relative strength at ages 5 and 6 increased to an unexpected degree. The effect of this is to substantially increase our estimates of this yearclass at younger ages, contrary to the indication of the survey." The possibility that the younger ages were outside the census area was not considered by these authors. Recently, the 1978 year-class at age 2 in Div. 4VW was estimated to be 8.9×10^6 fish (Zwanenburg 1989). This is approximately 40% below the long-term mean recruitment. The 1984 and 1985 year-classes in Div. 4VW were also more abundant when mature than when immature. These year-classes were subsequently determined to be slightly below average in abundance. These results are inconsistent with the dispersal model and the assumption of density-dependent emigration. Unfortunately, there are no reliable estimates of the interannual variability in the seasonal, geostrophic volume transport along the Scotian Shelf to assess whether hydrographic anomalies were associated with these exceptional years. It is not, there-

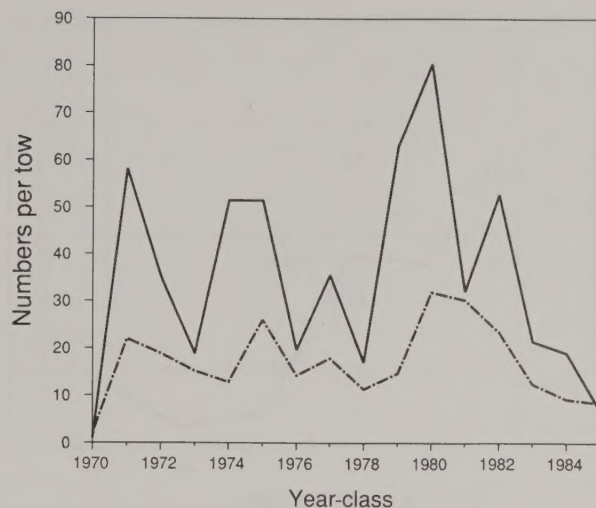


FIG. 6. Same as Fig. 5 except for Div. 4X haddock.

fore, possible to evaluate the contribution of alongshore post-larval transport to these patterns.

For the Div. 4X haddock, no year-classes were judged to be more abundant when mature (Fig. 6), although two year-classes (1970 and 1985) exhibited very similar abundances when immature and mature. Moreover, the magnitude of this difference between immature and mature indices was, on average, much greater in Div. 4X than in Div. 4VW: 35 and 17 haddock per standardized tow in Div. 4X versus 21 and 17 in Div. 4VW for immature and mature, respectively (Fig. 5 and 6). Expressed as instantaneous mortality (Z), Div. 4X haddock year-classes, on average, declined faster ($Z = 0.56$) than those in Div. 4VW ($Z = 0.06$). The hypothesized return migration of mature haddock from Div. 4X to Div. 4VW is consistent with the significant discrepancy between the average Z estimated for year-classes produced by the two stocks. There was no correlation between Div. 4X haddock year-class Z 's and haddock year-class strength in Div. 4VW. Perusal of the past assessment documents for Div. 4X haddock did not reveal any instances of year-classes having been revised upward to any great extent.

Analysis of the temporal dynamics of haddock year-classes, as derived from the RV surveys of the two stocks, suggests that the dispersal model is a viable representation of haddock stock dynamics on the Scotian Shelf.

Further support for the dispersal model is to be found in an analysis of growth rates in the two stocks. Assuming that emigration is density dependent (i.e. large year-classes emigrate more frequently than small ones) the carrying capacity for the immature ages in the upstream stock should seldom be reached. However, the return migration, associated with maturation of the former vagrants, will add to the resident stock and thus induce increased resource limitation among the older age groups. This leads to the expectation that the correlation between growth and population size for the immature age groups should be weak, while that for the mature ages should be stronger and negative. As predicted, the correlations between weight at age and population numbers at age for Div. 4VW haddock were weak and nonsignificant for the immature ages and strongly negative for mature fish (Fig. 7). A similar analysis for Div. 4X haddock yielded no significant correlations. However, in Div. 4X, there are strong spatial gradients in had-

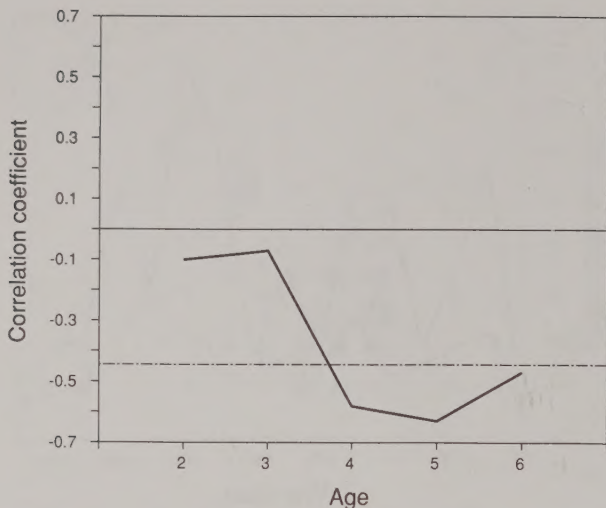


FIG. 7. Correlation coefficient for the relationship between population numbers at age and annual mean weight at age for Div. 4VW haddock. Both variables were transformed to natural logarithms prior to analysis. Data on population number and commercial weights at age taken from Zwanenburg (1989) and Zwanenburg and Comeau (1991), respectively. The broken line shows the significance level ($p < 0.05$).

dock size at age from the offshore banks to the approaches to the Bay of Fundy (O'Boyle et al. 1983) which may confound the growth response due to interannual differences in the stock distribution within Div. 4X. No such spatial variation in haddock size at age has been reported for Div. 4VW haddock.

Discussion

In their review of the problems that have plagued assessments of Atlantic herring stocks in the northwest Atlantic, Sinclair et al. (1985) seriously questioned whether management models applied to groundfish stocks were suitable for pelagic stocks. The available evidence on the spatial dynamics of haddock year-classes on the Scotian Shelf is consistent with the hypothesis of a unidirectional (possibly density dependent) mixing of the stocks during the pelagic juvenile stage, a mixed stock composition up to the age of maturation, and a return migration of adults (formerly vagrants) to their natal site. The pattern is also consistent with the known dispersal patterns of cod in Iceland and West Greenland, from which the present hypothesis was developed (Table 1). In the case of haddock, immigration and emigration cannot be safely ignored in population assessments. Clearly, these processes have important demographic consequences, although it is possible that the genetic identity of the two stocks would remain intact, given the hypothesized return migration at the age of maturity. Hence, the distinction drawn by Sinclair et al. (1985) on the differences between pelagic and groundfish should not be made until a more complete examination of other groundfish stock pairs is completed. Consistent with this conclusion, the tendency for the catchability (q) of pelagic stocks to increase with decreasing abundance (presumably due to increased aggregation at low population levels), once considered an exclusive characteristic of pelagic species, has now been shown to be true of groundfish species as well (haddock: Crecco and Overholtz 1990; hake (*Merluccius* spp.): Gordo and Duarte 1991; cod: Rose and Leggett 1991). It would be prudent, therefore, to enlarge the

haddock management unit on the Scotian Shelf to include both Div. 4X and 4VW stocks until the potential flux across stock boundaries is investigated and resolved. This recommendation is consistent with the management principles employed for pelagic species such as herring, where stock mixing during non-spawning periods is considered a characteristic feature of the species. The analyses presented above also suggest that attempts to link juvenile and adult stages through the generation of survival curves or life tables require a larger unit sampling area for juvenile stages relative to other life stages (Hourston 1959; Koeller et al. 1986).

The tracing of the development of the 1983 year-class for the two haddock stocks on the Scotian Shelf was problematic. For the downstream haddock stock (Div. 4X) the Koslow et al. (1985) prediction of year-class failure now appears to have been correct, in spite of the subsequent analyses which suggested otherwise (Campana et al. 1989; O'Boyle et al. 1989). My analyses suggest that the 1983 year-class in Div. 4X (which was largely based on its abundance at ages 0–3) represented a mixture of residents and immigrants weighted heavily towards the latter. Although estimation of the number of immigrants is not possible at this time, it is likely to have been large, given that the larval survey data document what was a reproductive failure on the part of Div. 4X haddock. These analyses suggest that the 1983 haddock year-class in Div. 4X should now be set to well below average, and previous analyses that concluded that it was the best year-class in the Fisheries Ecology Program time series (Smith et al. 1989) need revisiting. The factors influencing year-class strength, whether biological or physical, will obviously remain obscure with such confounded recruitment estimates. Only after cleansing the recruitment time series, through analysis of only those year-classes produced when upstream recruitment was average or below, or through proper accounting for individuals in a year-class (by enlarging the basic sampling unit), will valid tests of hypotheses concerning environmental regulation of year-class strength or stock/recruitment relationships be possible. Furthermore, the notion that larval surveys may be useful to predict year-class failures (Koslow et al. 1985) may indeed be valid, given my reanalysis of the 1983 haddock year-class in Div. 4X and the recent results of ichthyoplankton surveys for walleye pollock (*Theragra chalcogramma*) in the western Gulf of Alaska (Bailey and Spring 1992).

The conclusion that the hypothesized emigration was density dependent was not strongly supported by the haddock data. However, additional evidence from the literature (besides that for the Iceland and West Greenland cod stocks) shows that for strong year-classes the magnitude of spatial dispersion at the postlarval stage is high (haddock: Faroe Plateau (Saville 1965) and Georges Bank (Polacheck et al. 1992); sablefish (*Anoplopoma fimbria*): Bering Sea/Strait of Georgia (Beamish and McFarlane 1988); cod: Barents Sea (Sundby et al. 1989)). One of these studies (Polacheck et al. 1992) showed that the strong 1987 haddock year-class that originated on Georges Bank was distributed at the 0-group stage as far as 400 km beyond the normal limit of distribution, well into the mid-Atlantic Bight. This westward displacement was attributed to unusually strong along-shelf surface flows which, in turn, maintained bottom temperatures in the optimum range for 0-group haddock. Other strong year-classes were also associated with westward, off-bank distributions of 0-group fish that returned to Georges Bank as 1+ fish. This could be considered a spawning migration, given that the median age at maturity for male and female haddock on Georges Bank is 1.3 and 1.5 yr, respectively

(L. O'Brien, NMFS, Woods Hole, MA, unpubl. data). It is obvious from this example that spatial changes linked to abundance can also be rooted in environmental variability.

Sinclair (1988) has noted that populations of marine fishes are frequently observed to be mixed during nonspawning periods. Whether or not a significant temporal (interannual) component underlies this observation is largely unknown although it is generally believed that intermixing of stocks might explain coherence among strong year-classes but not weak ones (Grosslein 1987). I contend, on the basis of evidence available for cod (Iceland and West Greenland) and haddock (Scotian Shelf), that the conceptual framework underlying the population dynamics of marine fishes is in need of revision. Serious consideration should be given to developing and applying population models that explicitly acknowledge the demographic consequences of dispersal for both pelagic and groundfish stocks. This could immediately resolve the problem of underestimating the spatial scale necessary to correctly resolve the population dynamics. One such category of models, termed metapopulation models, is now considered to be an important tool for understanding distribution and abundance of organisms on large spatial scales (see review in Gotelli 1991). Such models generally assume that density-dependent emigration strongly influences the dynamics of both the receiving and source populations. The theoretical treatment of this subject by Pulliam (1988) is particularly relevant to current fisheries problems, some of which I have considered here. Pulliam (1988) argued that immigration from productive populations occupying "source" habitats can maintain large populations in nearby "sink" habitats. Populations in source habitats are defined as net exporters of individuals where local reproduction exceeds mortality and emigration rates exceed immigration. Conversely, populations in sink habitats are net importers of individuals, their reproductive rate is less than local mortality, and immigration exceeds emigration.

Without going into specific details of this demographic model, a summary of the model implications will highlight the utility of this framework for fisheries problems. If it is common for productive surpluses from source populations to immigrate into and maintain populations in sink habitats, then (1) studies of populations in sink habitats, given that a species may commonly occur and successfully reproduce in them, may mislead investigators about the habitat requirements of a species and yield little information on the factors regulating population size and (2) many species will occur in nature where conditions are not sufficient to maintain them without continued immigration (Pulliam 1988).

One fisheries problem that should be revisited in the light of the dispersal model is the issue of recruitment synchrony across stocks and between closely related species which has been argued to be evidence of the influence of large-scale environmental effects. For example, in the Gulf of Maine, three herring stocks have exhibited parallel time trends in recruitment. While mixing has been considered a possible explanation for synchronous strong year-classes (Anthony and Waring 1980), it has been generally disregarded in light of the observation of simultaneous weak year-classes. Positive correlations in recruitment among stocks distributed over broad spatial scales have been demonstrated for cod and haddock in the northwest Atlantic (Koslow 1984; Koslow et al. 1987). It has recently been determined, however, that the strongest correlations in year-class strength exist between neighbouring stocks of haddock and of cod (Cohen et al. 1991). While this result suggests that the dominant factors influencing recruitment are operating

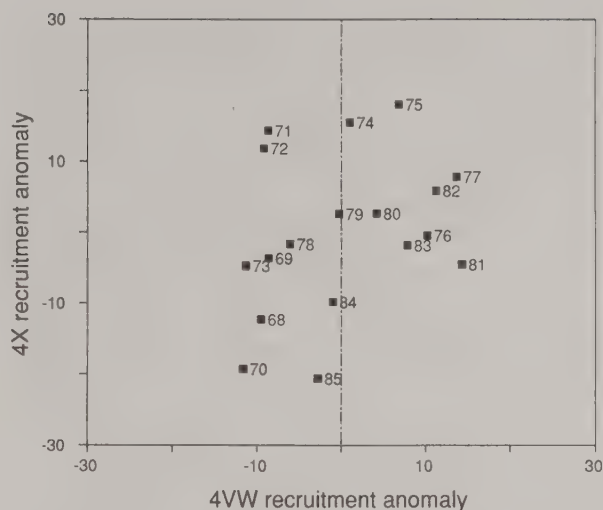


FIG. 8. Relationship between haddock recruitment in Div. 4X and 4VW, expressed as anomalies from the long-term mean. The broken line is centred on the mean for Div. 4VW haddock. Note: multiply units by 10^6 . Data taken from O'Boyle et al. (1989) and Zwanenburg (1989) for Div. 4X and Div. 4VW haddock, respectively.

on a more local scale, it could well be an exaggeration stemming from the groundfish management unit assumption. Density-dependent emigration could serve to minimize the difference in year-class strength between neighbouring stocks (as appears to have occurred for the 1983 haddock year-classes on the Scotian Shelf), the result being an artificially high correlation. In other words, the time series estimates of recruitment for the two stocks are not spatially independent. The development of methods to ensure spatial independence of recruitment estimates is critical to correctly determine the spatial scale of recruitment variability.

Iles and Sinclair (1982) and Sinclair (1988) have argued that the absolute abundance of marine populations appears to be, in part, a function of the geographic scale of the spawning and larval retention area. Of the various offshore banks supporting spawning stocks of haddock in the northwest Atlantic, Browns Bank in Div. 4X is by far the smallest (Browns Bank: 2247 km^2 ; Western Bank in Div. 4W: 4449 km^2 ; Georges Bank in Div. 5Z: 10221 km^2). Yet the long-term mean recruitment, averaged for the 1968–85 year-classes, was higher in Div. 4X (24.187×10^6 individuals at age 2) compared with Div. 4VW (15.038×10^6). The temporal variability in recruitment, expressed as the coefficient of variation (CV), was lower for Div. 4X haddock (45%) relative to Div. 4VW haddock (58%). This pattern of higher average recruitment and lower temporal variability for Div. 4X haddock is inconsistent with the prediction of Sinclair (1988). Can density-dependent emigration explain this exceptional pattern? To address this question, Fig. 8 was constructed in an attempt to isolate the assumed effect of density-dependent emigration on the Div. 4X haddock recruitment estimates. Calculating the mean and variance for those year-classes produced in Div. 4X when the Div. 4VW year-class anomalies were positive (emigration) and negative (no emigration) yielded a very different pattern. The mean recruitment in Div. 4X was lower and the variability higher ($\text{CV} = 56\%$) under the assumption of no emigration (i.e. left-hand side of Fig. 8). This result is more consistent with the expectations based on Sinclair's argument. Conversely, the temporal vari-

ability in Div. 4X recruitment was lower (CV = 26%) when emigration occurred (i.e. right-hand side of Fig. 8). Emigration could well be contributing to population growth and stability in Div. 4X haddock.

In an analogous situation, Dadswell (1979) has suggested that production among neighbouring stocks of lobsters is highly interdependent and maintained through linkages set up by residual currents which transport lobster larvae from one stock to another in a "domino effect" fashion. He reasoned that the dramatic decline in Chedabucto Bay lobster landings following the construction of the Canso causeway resulted from the disruption of the current system transporting lobster larvae from an upstream stock. Dadswell developed his conceptual model from early viewpoints on lobster recruitment mechanisms expressed by Scarratt (1964) and Iles (1975) who considered downstream stocks as those receiving additional recruitment from upstream stocks.

Finally, it is theoretically possible for emigration to act as a major factor regulating population density (Lidicker 1975). This could, in turn, influence the growth pattern. Sinclair (1988) put forth this same thought in a different manner, stating that "If vagrancy is at times density dependent, competition for resources is not required for the regulation of absolute abundance of marine populations." The pattern of density-dependent growth observed for the Div. 4VW haddock stock on the Scotian Shelf (Fig. 7) is entirely consistent with Sinclair's viewpoint and it suggests that emigration (or vagrancy) may be a possible mechanism permitting the regulation of population density below carrying capacity.

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References

ANONYMOUS. 1987. Report of the working group on cod stocks off east Greenland. ICES C.M. 1987/Assess:10.

ANTHONY, V. C., AND G. WARING. 1980. The assessment and management of the Georges Bank herring fishery. Rapp. P.-v. Réun. Cons. int. Explor. Mer 177: 72-111.

BAILEY, K. M., AND S. M. SPRING. 1992. Comparison of larval, age-0 juvenile and age-2 recruit abundance indices of walleye pollock, *Theragra chalcogramma*, in the western Gulf of Alaska. ICES J. Mar. Sci. 49: 297-304.

BEAMISH, R. J., AND G. A. MCFARLANE. 1988. Resident and dispersal behaviour of adult sablefish (*Anoplopoma fimbria*) in the slope waters off Canada's west coast. Can. J. Fish. Aquat. Sci. 45: 152-164.

BOLZ, G., AND R. LOUGH. 1988. Growth through the first six months of Atlantic cod, *Gadus morhua*, and haddock, *Melanogrammus aeglefinus*, based on daily otolith increments. Fish. Bull. U.S. 86: 223-236.

BOWEN, W. D. [ED.] 1987. A review of stock structure in the Gulf of Maine area: a workshop report. CAFSAC Res. Doc. No. 21: 51 p.

BUCH, E., AND H. H. HANSEN. 1986. Climate and cod fishery at West Greenland. Int. Symp. Long Term Changes Mar. Fish Popul. Vigo 1986: 345-364.

CAMPANA, S. E., K. T. FRANK, P. C. F. HURLEY, P. A. KOELLER, F. H. PAGE, AND P. C. SMITH. 1989. Survival and abundance of young Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) as indicators of year-class strength. Can. J. Fish. Aquat. Sci. 46(Suppl. 1): 171-182.

CLARK, J. R., AND V. D. VLADYKOV. 1960. Definition of haddock stocks of the northwestern Atlantic. U.S. Fish Wildl. Serv. Fish. Bull. 60: 283-296.

COHEN, E. B., D. G. MOUNTAIN, AND R. O'BOYLE. 1991. Local-scale versus large-scale factors affecting recruitment. Can. J. Fish. Aquat. Sci. 48: 1003-1006.

CRECCO, V., AND W. J. OVERHOLTZ. 1990. Causes of density-dependent catchability for Georges Bank haddock *Melanogrammus aeglefinus*. Can. J. Fish. Aquat. Sci. 47: 385-394.

DADSWELL, M. J. 1979. A review of the decline in lobster (*Homarus americanus*) landings in Chedabucto Bay between 1956 and 1977 with an hypothesis for a possible effect by the Canso Causeway on the recruitment mechanism of eastern Nova Scotia lobster stocks. Fish. Mar. Ser. Tech. Rep. 834.

DOUBLEDAY, W. G. [ED.] 1981. Manual on groundfish surveys in the northwest Atlantic. NAFO Sci. Coun. Stud. 2: 7-55.

DRINKWATER, K., B. PETRIE, AND W. H. SUTCLIFFE, JR. 1979. Seasonal geostrophic volume transports along the Scotian Shelf. Estuarine Coastal Mar. Sci. 9: 17-27.

FRANK, K. T., F. H. PAGE, AND J. K. MCRUER. 1989. Hydrographic effects on the vertical distribution of haddock (*Melanogrammus aeglefinus*) eggs and larvae on the southwestern Scotian Shelf. Can. J. Fish. Aquat. Sci. 46(Suppl. 1): 82-92.

GORDOA, A., AND C. M. DUARTE. 1991. Size-dependent spatial distribution of hake (*Merluccius capensis* and *Merluccius paradoxus*) in Namibian waters. Can. J. Fish. Aquat. Sci. 48: 2095-2099.

GOTELLI, N. J. 1991. Metapopulation models: the rescue effect, the propagule rain, and the core-satellite hypothesis. Am. Nat. 138: 768-776.

GROSSLEIN, M. D. 1987. Synopsis of knowledge of the recruitment process for Atlantic herring (*Clupea harengus*), with special reference to Georges Bank. NAFO Sci. Coun. Stud. 11: 91-108.

HALLIDAY, R. G., AND P. A. KOELLER. 1981. A history of Canadian groundfish trawling surveys and data usage in ICNAF Divisions 4TVWX. Can. Spec. Publ. Fish. Aquat. Sci. 58: 27-41.

HALLIDAY, R. G., AND A. T. PINHORN. 1990. The delimitation of fishing areas in the Northwest Atlantic. J. Northwest Atl. Fish. Sci. 10: 1-51.

HORSTED, S. A. 1989. Some features of oceanographic and biological conditions in Greenland water, p. 456-476. In L. Rey and V. Alexander [ed.] Proceedings of the sixth conference of the Comité Arctique International, 13-15 May 1985. E. J. Brill, Leiden, The Netherlands.

HOURSTON, A. S. 1959. The relationship of the juvenile herring stocks in Barkley Sound to the major adult herring populations in British Columbia. J. Fish. Res. Board Can. 16: 309-320.

HOVGÅRD, H., AND E. BUCH. 1990. Fluctuation in the cod biomass off the West Greenland Sea ecosystem in relation to climate, p. 36-43. In K. Sherman, L. M. Alexander, and B. D. Gold [ed.] Large marine ecosystems: patterns, processes, and yields. American Association for the Advancement of Science, Washington, DC.

HURLEY, P. C. F., AND S. E. CAMPANA. 1989. Distribution and abundance of haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) eggs and larvae in the waters off southwest Nova Scotia. Can. J. Fish. Aquat. Sci. 46(Suppl. 1): 103-112.

ILES, T. D. 1975. Analysis of the decline in the southern Gulf of St. Lawrence lobster landings to demonstrate differential area and time effects. ICES C. M. 1975/C:37: 16 p.

ILES, T. D., AND M. SINCLAIR. 1982. Atlantic herring: stock discreteness and abundance. Science (Wash., DC) 215: 627-633.

JONES, B. W. 1978. The potential contribution of cod from Greenland to the fishery at Iceland. ICES C.M. 1978/G:17.

KOELLER, P. A., P. C. F. HURLEY, P. PERLEY, AND J. D. NEILSON. 1986. Juvenile fish surveys on the Scotian Shelf: implications for year-class size assessments. J. Cons. int. Explor. Mer 43: 59-76.

KOSLOW, J. A. 1984. Recruitment patterns in northwest Atlantic fish stocks. Can. J. Fish. Aquat. Sci. 41: 1722-1729.

KOSLOW, J. A., S. BRAULT, J. DUGAS, AND F. PAGE. 1985. Anatomy of an apparent year-class failure: the early life history of the 1983 Browns Bank haddock *Melanogrammus aeglefinus*. Trans. Am. Fish. Soc. 114: 478-489.

KOSLOW, J. A., K. R. THOMPSON, AND W. SILVERT. 1987. Recruitment to northwest Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) stocks: influence of stock size and climate. Can. J. Fish. Aquat. Sci. 44: 26-39.

LIDICKER, W. Z. JR. 1975. The role of dispersal in the demography of small mammals, p. 103-128. In F. B. Golley, K. Petrusiewicz, and L. Ryszkowski [ed.] Small mammals: their productivity and population dynamics. Cambridge University Press, New York, NY.

1985. An overview of dispersal in non-volant small mammals. Contrib. Mar. Sci. 27(Suppl.): 369-385.

LODER, J. W., C. K. ROSS, AND P. C. SMITH. 1988. A space- and time-scale characterization of circulation and mixing over submarine banks, with

- application to the northwestern Atlantic continental shelf. *Can. J. Fish. Aquat. Sci.* 45: 1860–1885.
- MAHON, R., P. SIMPSON, AND D. E. WALDRON. 1985. The eastern Scotian Shelf (4VW) haddock stock and fishery in 1984, with an historical perspective on stock and recruitment back to 1948. *CAFSAC Res. Doc.* 85/47: 62 p.
- O'BOYLE, R. N., K. T. FRANK, AND J. SIMON. 1989. An evaluation of the population dynamics of 4X haddock during 1962–88 with yield projected to 1990. *CAFSAC Res. Doc.* 89/95: 62 p.
- O'BOYLE, R. N., M. SINCLAIR, R. J. CONOVER, K. H. MANN, AND A. C. KOHLER. 1984. Temporal and spatial distribution of ichthyoplankton communities of the Scotian Shelf in relation to biological, hydrological, and physiographic features. *Rapp. P.-v. Réun. Cons. int. Explor. Mer* 183: 27–40.
- O'BOYLE, R. N., K. WAIWOOD, AND J. McMILLAN. 1983. An evaluation of the 4X haddock population characteristics during 1962–1982 with yield projected to 1984. *CAFSAC Res. Doc.* 83/73: 52 p.
- PAGE, F. H., AND K. T. FRANK. 1989. Spawning time and egg stage duration in northwest Atlantic haddock stocks with emphasis on Georges and Browns Bank. *Can. J. Fish. Aquat. Sci.* 46(Suppl. 1): 68–81.
- POLACHECK, T., D. MOUNTAIN, D. McMILLAN, W. SMITH, AND P. BERRIEN. 1992. Recruitment of the 1987 year class of Georges Bank haddock (*Melanogrammus aeglefinus*): the influence of unusual larval transport. *Can. J. Fish. Aquat. Sci.* 49: 484–496.
- PULLIAM, H. R. 1988. Sources, sinks and population regulation. *Am. Nat.* 132: 652–661.
- ROSE, G. A., AND W. C. LEGGETT. 1991. Effects of biomass–range interactions on catchability of migratory demersal fish by mobile fisheries: an example of Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* 48: 843–848.
- SAVILLE, A. 1965. Factors controlling dispersal of the pelagic stages of fish and their influence on survival. *ICNAF Spec. Publ.* 6: 335–348.
- SCARRATT, D. J. 1964. Abundance and distribution of lobster larvae (*Homarus americanus*) in Northumberland Strait. *J. Fish. Res. Board Can.* 21: 661–679.
- SCHOPKA, S. A. 1991. The Greenland cod at Iceland 1941–1990 and its impact on assessment. *NAFO SCR Doc.* 91/102, Ser. No. N1994: 7 p.
- SINCLAIR, M. 1988. Marine populations: an essay on population regulation and speciation. University of Washington Press, Seattle, WA. 252 p.
- SINCLAIR, M., V. C. ANTHONY, T. D. ILES, AND R. N. O'BOYLE. 1985. Stock assessment problems in Atlantic herring (*Clupea harengus*) in the north-west Atlantic. *Can. J. Fish. Aquat. Sci.* 42: 888–898.
- SISSENWINE, M. P. 1981. An overview of some methods of fish stock assessment. *Fisheries* 6: 31–35.
- SMITH, P. C., K. T. FRANK, AND R. MAHON. 1989. General introduction to southwest Nova Scotia Fisheries Ecology Program 1982–1989. *Can. J. Fish. Aquat. Sci.* 46(Suppl. 1): 2–3.
- SMITH, S. J., AND G. D. SOMERTON. 1981. STRAP: a user-oriented computer analysis system for groundfish research trawl survey data. *Can. Tech. Rep. Fish. Aquat. Sci.* 1030: iv + 66 p.
- SUNDBY, S., H. BJØRKE, A. V. SOLDAL, AND S. OLSEN. 1989. Mortality rates during the early life stages and year-class strength of northeast Arctic cod (*Gadus morhua* L.). *Rapp. P.-v. Réun. Cons. int. Explor. Mer* 191: 351–358.
- TEMPLEMAN, W., AND C. A. BISHOP. 1979. Age, growth, year-class strength, and mortality of haddock, *Melanogrammus aeglefinus*, on St. Pierre Bank in 1948–75 and their relation to the haddock fishery of this area. *ICNAF Res. Bull.* 14: 85–99.
- TEMPLEMAN, W., V. M. HODDER, AND R. WELLS. 1978. Age, growth, year-class strength, and mortality of the haddock, *Melanogrammus aeglefinus*, on the southern Grand Bank and their relation to the haddock fishery of this area. *ICNAF Res. Bull.* 13: 31–52.
- WAIWOOD, K. G., AND M.-I. BUZETA. 1989. Reproductive biology of South-west Scotian Shelf haddock (*Melanogrammus aeglefinus*). *Can. J. Fish. Aquat. Sci.* 46(Suppl. 1): 153–170.
- WYNNE-EDWARDS, V. C. 1962. Animal dispersion in relation to social behavior. Oliver and Boyd, Edinburgh, Scotland.
- ZWANENBURG, K. 1989. Assessment of 4TVW haddock with catch projections to 1990. *CAFSAC Res. Doc.* 89/64: 36 p.
1992. An assessment of eastern Scotian Shelf haddock for 1992. *CAFSAC Res. Doc.* 92/39: 42 p.
- ZWANENBURG, K., AND P. COMEAU. 1991. Haddock on the eastern Scotian Shelf. *CAFSAC Res. Doc.* 91/47: 43 p.

Onshore Movements of Atlantic Mackerel (*Scomber scombrus*) in the Northern Gulf of St. Lawrence: Associations with Wind-Forced Advections of Warmed Surface Waters¹

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We employed fisheries acoustic techniques to assess the distribution and relative abundance of Atlantic mackerel (*Scomber scombrus*) at high resolution at Brador Bay in the northern Gulf of St. Lawrence in 1985 and 1986. These data were used to test the hypotheses that (1) onshore movements of mackerel are associated with wind-forced advections of heated surface waters and (2) mackerel are confined to waters having temperatures $\geq 7^{\circ}\text{C}$. Increased mackerel densities or "mackerel events" followed landward advections of heated surface waters in both 1985 and 1986. Landward advections of surface waters, and mackerel events, followed alongshore wind stress from the northeast. In our daytime observations, mackerel tended to concentrate in waters having temperatures of approximately 4°C , near bottom, beneath warmer surface waters. However, the overall probability of mackerel occurrence inshore was much greater when near-bottom waters were warmer ($\geq 7^{\circ}\text{C}$). Mackerel densities were not correlated with salinities, cross-shore winds, or currents but were negatively correlated with Atlantic cod (*Gadus morhua*) densities within the study site.

Nous avons utilisé les techniques d'acoustique halieutique pour étudier la distribution et l'abondance à haute résolution du maquereau bleu (*Scomber scombrus*) dans la Baie de Brador (nord du golfe du Saint-Laurent). Ces données ont servi à tester les hypothèses que 1) les mouvements du maquereau vers la côte sont associés à l'advection par le vent d'eaux chaudes de surface et 2) le maquereau est confiné à des eaux de température $\geq 7^{\circ}\text{C}$. Les augmentations de densité de maquereau ou les "événements de maquereau" de 1985 et de 1986 ont suivi l'advection vers la côte d'eau chaude de surface. L'advection vers la côte d'eau chaude et les événements de maquereau ont suivi des conditions de contrainte de vent parallèle à la rive du nord-est. Nos observations diurnes indiquent que le maquereau était surtout concentré dans des eaux à 4°C , près du fond, sous des eaux de surface plus chaudes. Cependant, la probabilité d'occurrence du maquereau près de la rive était beaucoup plus élevée lorsque l'eau près du fond était $\geq 7^{\circ}\text{C}$. Les densités de maquereau dans la baie de Brador n'étaient pas corrélées aux salinités, aux vents perpendiculaires à la rive ou aux courants mais étaient négativement corrélées aux densités de la morue franche (*Gadus morhua*).

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Scombrids have been hypothesized to migrate by a process termed "enviroregulation," the behavioral regulation of immediate environmental conditions (Neill 1984). For example, Atlantic mackerel (*Scomber scombrus*) are thought to conduct springtime inshore and northward migrations along the 7°C sea surface isotherm (e.g., Sette 1950; Jackman and Steven 1955; Ware and Lambert 1985). In keeping with this hypothesis, Olla et al. (1975) found that captive mackerel increased their swimming speed at water temperatures of $<7^{\circ}\text{C}$. This result was interpreted as a behavioral avoidance of nonpreferred temperatures.

Spawning of the "northern contingent" of mackerel (Sette 1950) in the Northwest Atlantic is primarily concentrated over

the Magdalen Shallows (southern Gulf of St. Lawrence) from mid-June to early July (Sette 1943; Ouellet 1987). After spawning, part of the stock migrates into the northerly reaches of the Gulf of St. Lawrence and along the eastern shores of Newfoundland (Moore et al. 1975). During these feeding migrations, mackerel have been reported to occur as far north as coastal Labrador (Parsons 1970). Onshore movements of mackerel in the northern Gulf of St. Lawrence have been hypothesized to coincide with wind-driven advections of warmed surface waters (Rose and Leggett 1988b).

The occurrence of Atlantic cod (*Gadus morhua*) on the north shore of the Gulf of St. Lawrence often coincides with thermal variations caused by the coastal upwelling of cold, deep Gulf waters (Rose and Leggett 1988c). Under the hypothesis that mackerel movements are associated with the advection of warmed surface waters, the occurrence of cod and mackerel should be negatively correlated on this coast. Consistent with this hypothesis, fishermen along the north shore of the Gulf of

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St. Lawrence have long claimed that "when the mackerel come ashore, the cod will soon be gone."

In this study, we tested two related hypotheses on the movements of mackerel relative to thermal conditions in the northern Gulf of St. Lawrence: (1) that increases of mackerel densities inshore are associated with onshore advection of warm surface waters and (2) that mackerel are confined to waters having temperatures $\geq 7^{\circ}\text{C}$. We also tested the hypothesis that, as a consequence of the inverse effects of coastal temperatures on cod and mackerel, the densities of these two species would be negatively correlated in nearshore waters.

Materials and Methods

The study was conducted at Brador Bay ($51^{\circ}25'\text{N}$, $57^{\circ}15'\text{W}$) in the northern Gulf of St. Lawrence (Fig. 1). The study area encompasses about 250 km^2 (30 km of coastline by 5–8 km offshore). Maximum water depth in the study area is approximately 100 m. Full details of the hydrography and physical oceanography in the study area are given in Rose and Leggett (1988b, 1988c, 1989a, 1989b). Mackerel densities within Brador Bay were surveyed daily from July 12 to August 1, 1985, and from June 13 to August 4, 1986, using the methods of fisheries acoustics (Mitson 1983). All acoustic measurements were made at 120 kHz with a 10° transducer housed in a "V-fin" towed 1–2 m below the sea surface at $10\text{ km}\cdot\text{h}^{-1}$ from a 10-m vessel. Relative density estimates were obtained by employing standard echo integration techniques (Burczynski 1982). Mackerel echoes were distinguished from capelin (*Mallotus villosus*) and cod echoes using the quadratic discriminant functions developed for that purpose by Rose and Leggett (1988a).

An average of 43 (range 21–66) and 50 (range 21–116) 1-km transects were run daily in 1985 and 1986, respectively. The overall survey design was systematic and fixed. Transects

were run across the study area in zigzags designed to sample all spatial and depth ranges. Precise transects were initially chosen at random. All transects were run between 06:00 and 16:00 (EDT). Acoustic density estimates (volts squared) were calculated for each 10-m depth stratum of every 1-km transect. To allow for comparisons with meteorological and oceanographic variables, acoustic densities over all depth strata and locations were averaged by day. Further details regarding the acoustic methods and the analyses of data are provided in Rose and Leggett (1988a).

The acoustic densities presented must be considered as relative indices of mackerel abundance within the study area over time. Absolute abundance estimates have not been attempted for two reasons: (1) horizontal avoidance of the boat almost certainly limited the encounter rate (we assumed that this bias was constant) and (2) the difficulty of determining a correct mean backscattering cross-section for this species (Rose and Leggett 1988a).

Water temperatures were assessed along the acoustic transects using two methods: (1) near-surface temperature was measured periodically with a mercury thermometer in 1985 and continuously with a Ryan Tempmentor thermistor (Ryan Corp., WA) fastened to the acoustic towed body in 1986 and (2) an average of three expendable bathythermograph (XBT, Sippican Corp., MA) profiles were recorded daily at variable locations in both 1985 and 1986. Temperatures were also measured by 16 recording thermographs moored in the study area (details of placement are given in Rose and Leggett 1988b). A sea temperature value was assigned to each 10-m-deep by 1-km-long acoustic transect stratum on the basis of interpolations of temperatures from XBT profiles, near-surface measurements, and the thermographs. Mean daily near-surface (0–10 m) temperatures in Brador Bay were also calculated from the above temperature measurements. Rose and Leggett (1989a) verified

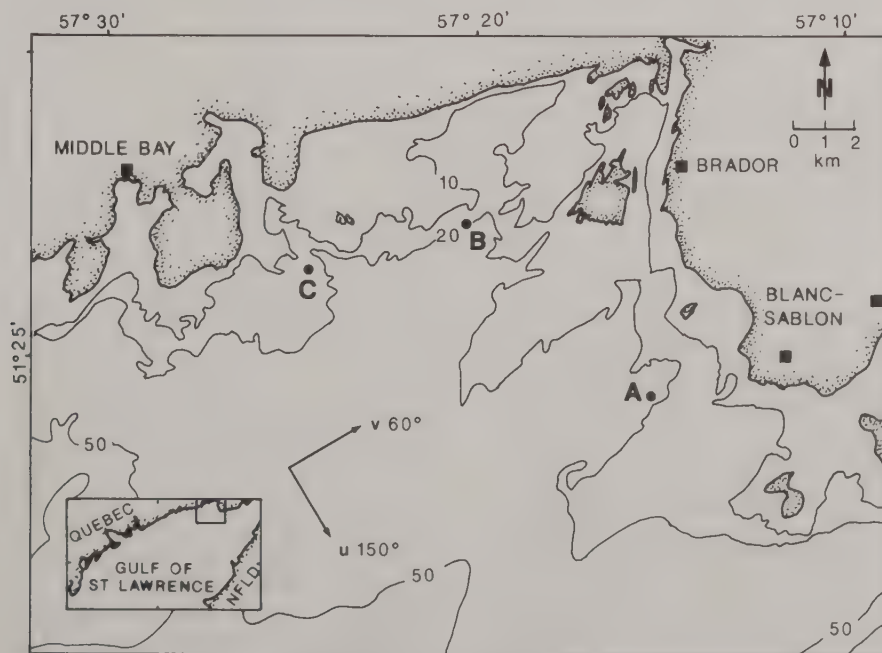


FIG. 1. Brador Bay area, showing location of current meters (A, B, C) and the v and u components for currents. Isobaths are in fathoms (1 fathom = 1.8 m).

temperature interpolations against actual XBT profiles and found near-bottom and near-surface interpolations to be accurate within $\pm 1^\circ\text{C}$.

In addition to the in situ temperature measurements described above, water currents, temperatures, and salinities within the study site were continuously monitored with six Aanderaa RCM4 current meters moored at middepth and close to bottom at three sites (Fig. 1). Current meter depths averaged over the two years were 18 and 31 m for site A (mean water depth 52 m), 21 and 35 m for site B (mean water depth 37 m), and 20 and 32 m for site C (mean water depth 33 m). In terms of current directions and sea temperatures, site A, located beyond the outer boundary of Brador Bay, is characteristic of offshore conditions, while sites B and C are characteristic of nearshore conditions at Brador Bay (Rose and Leggett 1988b). The time series of current, temperature, and salinities were low-pass filtered to remove periodicities < 24 h (Godin 1972). Current direction (of travel) and velocity were resolved as alongshore ($+v = 60^\circ\text{true}$) and cross-shore ($+u = 150^\circ\text{true}$). Daily temperature and current means were then calculated from 17:00 to 16:00 for comparison with the mean daily acoustic densities.

Hourly wind direction (of origin) and velocity data were obtained from the Blanc Sablon weather station, located approximately 50 km east of Brador Bay. Wind was decomposed into alongshore ($+v = 60^\circ\text{true}$) and cross-shore ($+u = 330^\circ\text{true}$) components. For wind speeds $\leq 7 \text{ m}\cdot\text{s}^{-1}$, alongshore and cross-shore wind speeds were transformed into wind stresses according to Csanady (1982):

$$(1) F_y = 2 \times 10^{-6} W \cdot W_y$$

where F_y is the wind stress along axis y (square metres per second squared) W is the wind speed (metres per second), and W_y is the wind vector along axis y (metres per second). For $W > 7 \text{ m}\cdot\text{s}^{-1}$, appropriate equations shown in Csanady (1982, p. 11) were used to calculate the wind stress. Daily wind stress averages (17:00–16:00) were then calculated for comparison with the mean daily acoustic densities.

Mean daily mackerel density estimates were correlated with mean daily sea temperatures near the surface (0–10 m), at mid-depth (18–21 m), and close to bottom (31–35 m). Mean daily density estimates were also correlated with wind stresses, salinities, currents, and cod densities. Nonparametric procedures were used because mean daily mackerel acoustic densities departed significantly from a normal distribution (Kolmogorov-Smirnov test, $P < 0.001$).

Results

Meteorological and Oceanographic Conditions

Rose and Leggett (1988b) provided a detailed description of the relationships among winds, currents, and sea temperatures at Brador Bay. A brief overview of that analysis as it pertains to this study is presented here.

During the summer of 1985, currents and sea temperatures in Brador Bay (tidal variations removed) were coherent with alongshore wind stress at periods of > 2 d and with cross-shore wind stress at shorter periods. Prevailing summer winds were alongshore from the southwest in 1985, which represented a typical summer wind pattern for the area (Vigeant 1984). The persistence of these winds forced northeastern currents and upwelling of cold, deep, offshore Gulf waters along the coast. Upwellings caused thermocline elevations and corresponding declines in nearshore temperatures of up to 10°C within 24 h. The relaxation of upwelling following the onset of strong winds

from the northeast generated the opposite effect, forcing southwestern currents and the onshore movement and coastal downwelling of warmer surface waters.

Atmospheric and oceanic conditions at Brador Bay differed between 1985 and 1986 (Rose and Leggett 1989b). During the summer of 1985, winds were predominantly from the northeast. The winds from the southwest that did occur were of insufficient strength and duration to generate upwelling conditions (with the exception of a minor upwelling event between days 203 and 206). In contrast with hydrographic conditions in 1985, heated surface waters circulating from the eastern side of the Gulf, mixed with Labrador Sea waters inflowing through the Strait of Belle Isle, dominated the nearshore waters in the northwest Gulf throughout most of the summer in 1986.

Mackerel Movements into Brador Bay

Over the two summers of study at Brador Bay, three main periods of increased mackerel densities (hereafter referred to as "mackerel events") were observed. They occurred on days 202–205 (July 21–24) and on days 209–213 (July 28 to August 1) in 1985 (Fig. 2) and on days 209–216 (July 28 to August 4) in 1986 (Fig. 3). The mackerel events occurred after spawning, during the ensuing feeding migration. Mackerel were encountered in the study area on 11 of 17 sampling days in 1985 and on 21 of 44 sampling days in 1986.

In both 1985 and 1986, each mackerel event occurred after rapid increases in nearshore sea temperatures related to the relaxing of upwelling and the onset of downwelling conditions (Fig. 2 and 3). Mean daily mackerel densities and Brador Bay near-surface temperatures were positively correlated in both 1985 and 1986 (Spearman's r_s , $P < 0.005$) (Table 1; Fig. 2 and 3). Mean daily mackerel densities were also positively correlated with middepth and bottom sea temperatures in both Brador Bay and offshore in 1985 and with middepth sea temperatures in both Brador Bay and offshore in 1986 (Spearman's r_s , $P < 0.03$) (Table 1). In 1986, mackerel densities were most strongly correlated with Brador Bay near-surface temperatures and offshore middepth temperatures on the day prior to the acoustic survey (Spearman's r_s , $P < 0.01$) (Table 1).

Relationships between alongshore winds, sea temperatures, and mackerel densities were evident in both 1985 and 1986. In 1985, strong winds from the northeast on days 197 and 198 were followed by nearshore temperature increases of 5°C (from 6 to 11°C) on days 200–204. This period of elevated nearshore temperatures coincided with the first mackerel event (Fig. 2). Alongshore winds from the southwest on days 204–206 were followed by strong upwelling conditions along the north shore and at Brador Bay on days 205–208 (Rose and Leggett 1988b). The relaxing of this upwelling, as a consequence of slackening of winds from the southwest, led to rapid temperature increases as warmed surface waters replaced cooler upwelled waters nearshore. This advancement of warmed surface waters coincided with the second mackerel event in 1985 (Fig. 2).

Among the winds from the northeast that predominated along the north shore of the Gulf during the summer of 1986, two periods of stronger winds can be identified (Fig. 3). The first, between days 180 and 186, coincided with increased nearshore surface temperatures (from 2 to 9°C). The relatively small runs of mackerel that occurred during and following this early warming suggest that mackerel had not yet arrived in the northern Gulf from their southerly spawning grounds. The second period of stronger winds from the northeast occurred from day 210 to 213 near the end of the study period in 1986. This period coincided with the rapid warming of the nearshore surface waters

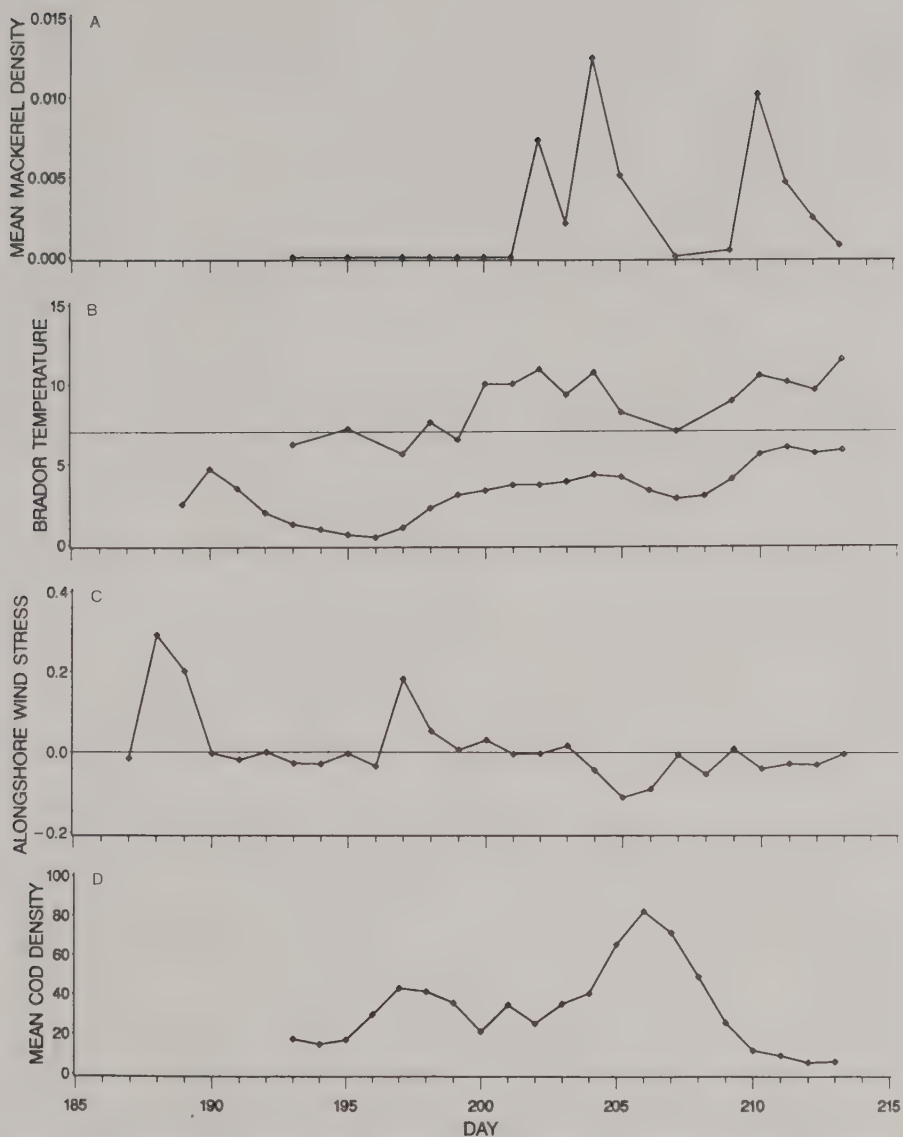


FIG. 2. (A) Mean daily relative acoustic densities (V^2) of mackerel in Brador Bay in 1985, (B) Brador Bay near-surface (top line) and bottom water temperatures ($^{\circ}\text{C}$; 7°C water is shown for reference), (C) alongshore wind stress ($10^{-3} \text{ m}^2 \cdot \text{s}^{-2}$; values above the line indicate wind stress from the northeast), and (D) mean daily absolute acoustic density of cod (number of fish per 10^5 m^3) in Brador Bay.

(from 5 to 13°C) and with the single mackerel event recorded in 1986.

In 1986, mean daily mackerel densities were positively correlated with alongshore wind stress from the northeast on the previous day (Spearman's $r_s = 0.35$, $P < 0.03$) (Fig. 3) and 2 d prior to the acoustic survey (Spearman's $r_s = 0.45$, $P < 0.003$) (Fig. 3). In 1985, the sign of the correlation was reversed: mean daily mackerel densities in Brador Bay were negatively correlated with alongshore wind stress from the northeast on the same day (Spearman's $r_s = -0.63$, $P < 0.007$) (Fig. 2) but not on previous days.

Mean daily mackerel densities in Brador Bay were negatively correlated with mean daily cod densities in 1986 (Spearman's $r_s = -0.33$, $P < 0.05$) (Fig. 3) but not in 1985 (Spearman's r_s , $P > 0.05$) (Fig. 2). Correlations of mean daily mackerel densities with salinities were not significant for either year

(Spearman's r_s , $P > 0.05$), with the single exception of a correlation with offshore bottom salinity in 1985 (Spearman's $r_s = -0.70$, $P < 0.002$). Cross-shore wind stresses and alongshore and cross-shore currents were not significantly correlated with mean daily mackerel densities in 1985 or 1986 (Spearman's r_s , $P > 0.05$).

Mackerel Spatial Distribution and Temperature

Mackerel commonly occurred over sea temperatures ranging from 3 to 13°C (the maximum observed) in both 1985 and 1986 (Fig. 4). The sea temperature at which mackerel occurred most frequently was 4°C in 1985 and 5°C in 1986. In 1986, mackerel were occasionally located in waters as cold as 0°C . These findings are inconsistent with our second hypothesis, that mackerel would be confined to waters having temperatures $\geq 7^{\circ}\text{C}$. In the

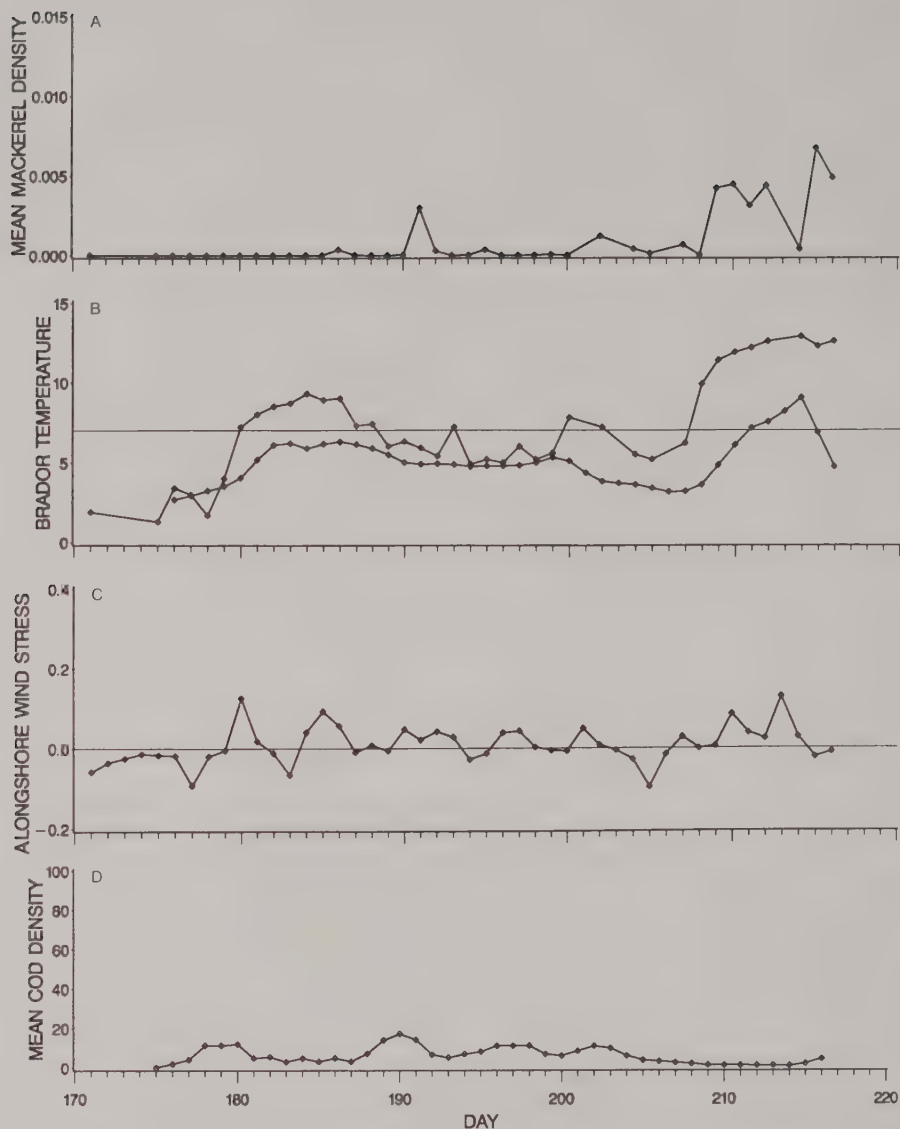


FIG. 3. (A) Mean daily relative acoustic densities (V^2) of mackerel in Brador Bay in 1986, (B) Brador Bay near-surface (top line) and bottom water temperatures ($^{\circ}\text{C}$; 7°C water is shown for reference), (C) alongshore wind stress ($10^{-3} \text{ m}^2 \cdot \text{s}^{-2}$; values above the line indicate wind stress from the northeast), and (D) mean daily absolute acoustic density of cod (number of fish per 10^5 m^3) in Brador Bay.

TABLE 1. Spearman's coefficients of rank correlation (with P in parentheses) between mean daily mackerel acoustic densities and mean daily water temperatures. Densities are correlated with sea temperatures on the same day, except for Brador Bay near-surface, and offshore mid-depth temperatures in 1986 for which correlations with temperatures on the day prior to sampling are shown. No offshore near-surface temperature series was available. n.s., not significant.

Location	1985	1986
Brador Bay, near-surface	0.67 ($P = 0.004$)	0.59 ($P = 0.0001$)
Brador Bay, middepth	0.67 ($P = 0.003$)	0.36 ($P = 0.03$)
Brador Bay, bottom	0.76 ($P = 0.0004$)	n.s.
Offshore, middepth	0.64 ($P = 0.006$)	0.40 ($P = 0.01$)
Offshore, bottom	0.79 ($P = 0.0002$)	n.s.

two years, the observed distribution of mackerel relative to water temperature differed significantly from the total distribution of temperatures sampled (Kolmogorov-Smirnov tests, $P < 0.0001$) (Fig. 4).

Total acoustic densities of mackerel were greatest in waters having temperatures of 4°C in both 1985 and 1986 (Fig. 5A). In contrast, mean densities peaked in waters having temperatures of 7 and 11°C in 1985 and 1986, respectively (mean density is defined as the total density for a given temperature divided by the number of times that temperature was observed during the course of the study) (Fig. 5B). Furthermore, the probability of observing mackerel was much greater in waters having temperatures of $\geq 7^{\circ}\text{C}$ in both 1985 and 1986 (Table 2). Percent occurrence of mackerel per degree of temperature peaked at 11 and 12°C in both 1985 and 1986. For example,

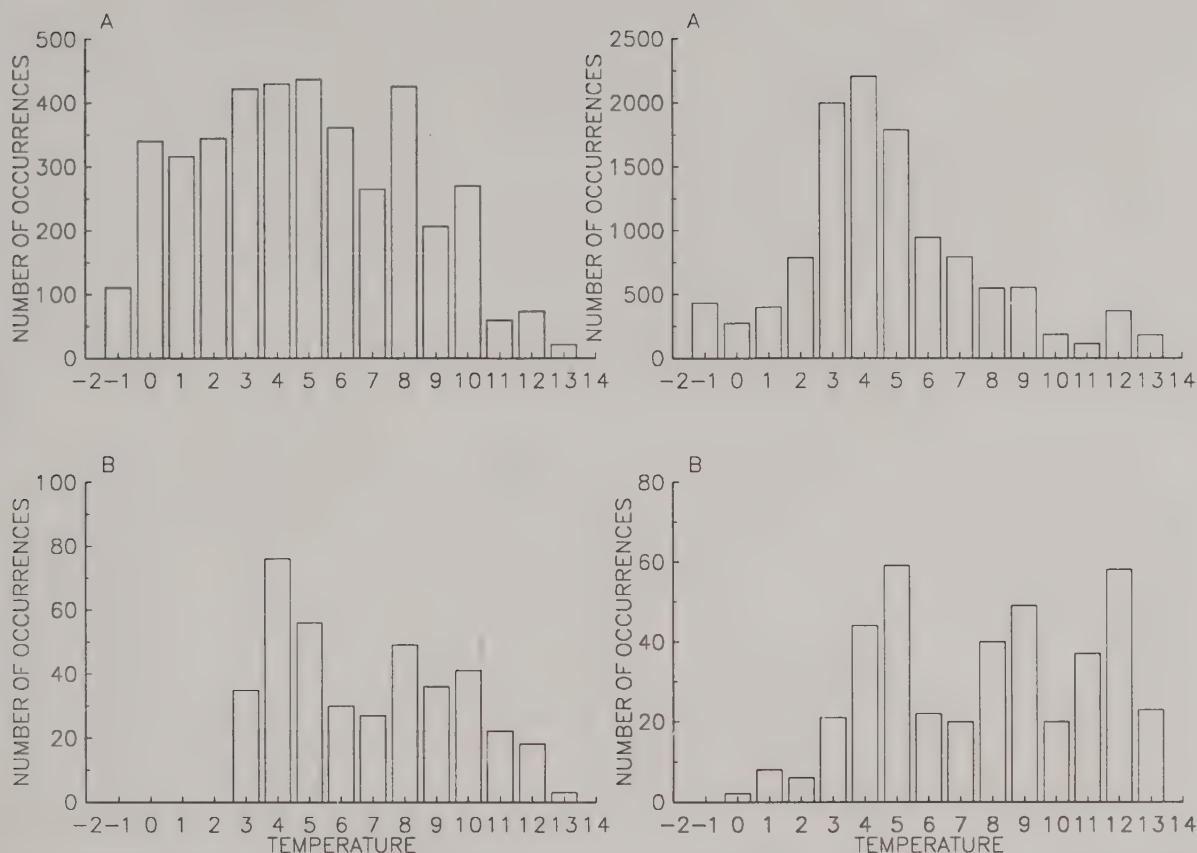


FIG. 4. (A) Observed occurrences of temperatures in Brador Bay in 1985 and 1986 for all 10-m depth strata and (B) number of times that mackerel were observed at each temperature for all depth strata.

the estimated probability of observing mackerel in Brador Bay in July 1985 rose from 9% at 3°C to 39% at 11°C (Table 2). We also observed a secondary peak of percent occurrence at 4°C in 1985. The lower overall percentage occurrences in 1986, as compared with 1985, may be explained by the earlier sampling in 1986, when mackerel were not observed in Brador Bay, and also by the fact that mackerel daily densities were generally larger in 1985 (Fig. 2) than in 1986 (Fig. 3).

Finally, we examined the vertical distribution of mackerel relative to thermal conditions above and below the strong thermocline which develops during the summer at Brador Bay (Fig. 6). The greatest mackerel densities were found between 15 and 35 m deep (Fig. 7), with mean depths and distances off bottom (weighted by acoustic densities) of 15.2 and 7.5 m in 1985 and 14.6 and 3.0 m in 1986, respectively. In both 1985 and 1986, mackerel occurred most frequently in 4°C water when near-surface temperatures (NST) were $\geq 7^\circ\text{C}$. In 1985, almost all mackerel occurrences (96%) were recorded when $\text{NST} \geq 7^\circ\text{C}$ whereas 81% of transects had $\text{NST} \geq 7^\circ\text{C}$. In 1986, 78% of mackerel occurrences were when $\text{NST} \geq 7^\circ\text{C}$ whereas only 51% of transects had $\text{NST} \geq 7^\circ\text{C}$. For both years, mackerel were associated with bodies of water having $\text{NST} \geq 7^\circ\text{C}$ (relative to waters having $\text{NST} < 7^\circ\text{C}$, χ^2 tests, $P < 0.0001$). This association of mackerel with waters having $\text{NST} \geq 7^\circ\text{C}$ is also evident in Fig. 2 and 3.

Discussion

Our data indicate that mackerel movements into Brador Bay are associated with onshore advections of warmed ($\geq 7^\circ\text{C}$) surface waters, as hypothesized by Rose and Leggett (1988b). These onshore advections of warm surface waters in the northern Gulf (downwelling) are known to be caused by the relaxing of upwelling conditions induced by alongshore winds from the northeast or by slackening of winds from the southwest (Rose and Leggett 1988b). We found cooccurrence of downwelling periods and mackerel events in both 1985 and 1986. We also observed in 1986 a maximum positive correlation between mackerel densities and alongshore wind stress from the northeast 2 d prior to the acoustic survey. The absence of relationships between mackerel densities and cross-shore winds, currents, and salinities (except in one case) argues for the primary importance of alongshore wind-forced thermal advections in regulating nearshore distribution of mackerel in Brador Bay. Our findings are in agreement with Dannevig's (1959, cited in Lindquist and Hannerz 1974) observations that, in the North Sea, "if this warm [surface] water is driven from the coast by the wind, the mackerel also disappear from the coast." Moreover, Overholtz et al. (1991) reported that the magnitude of mackerel catch in a spring recreational fishery may be influenced by wind direction and intensity.

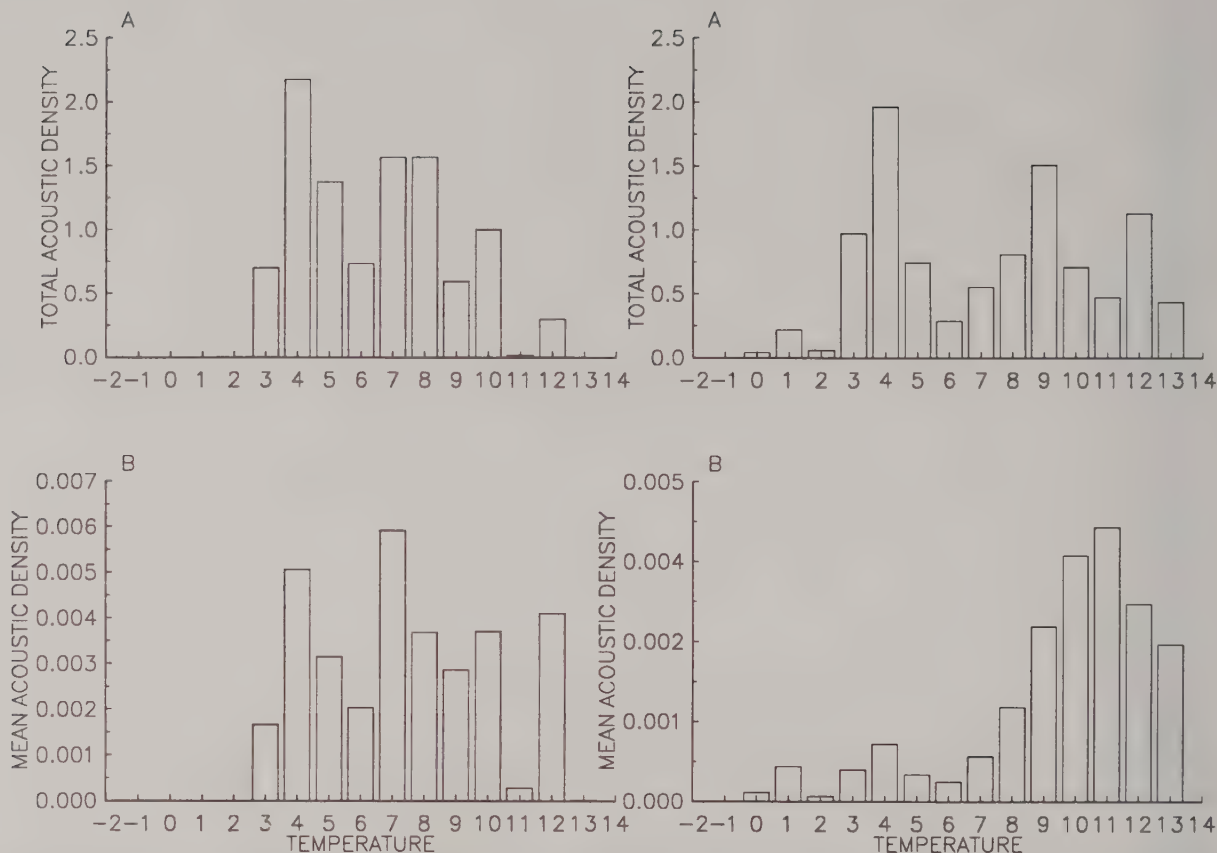


FIG. 5. (A) Total relative mackerel acoustic density (V^2) observed per degree Celsius of water temperature for all 10-m depth strata and (B) mean relative mackerel acoustic density (V^2) per degree Celsius of water temperature for all depth strata in 1985 and 1986. Mean density is defined as the total density for a given temperature divided by the number of times that temperature was observed.

TABLE 2. Percent occurrence of mackerel per degree Celsius of temperature in 1985 and 1986. Percent occurrence is defined as the number of times mackerel was observed at a given temperature divided by the number of times that temperature was observed.

Temperature (°C)	1985	1986
-1	0	0
0	0	0.7
1	0	2.0
2	0	0.8
3	8.5	1.1
4	18.8	2.0
5	14.0	3.3
6	9.1	2.3
7	10.6	2.5
8	12.2	7.3
9	17.4	8.9
10	15.6	10.9
11	39.0	33.6
12	27.4	15.8
13	14.3	12.9

Thermocline dynamics could also influence mackerel movements into Brador Bay. For example, a strong thermocline at a depth of 15–20 m (similar to the mean depth of mackerel in this study) was present during the first mackerel event of 1985

(Fig. 6: days 202–205) but became much more diffuse during the following cooling period induced by winds from the southwest (Fig. 6: days 207–209). Mackerel events also occurred when the difference between near-surface and bottom temperatures was strongest. Mackerel could be aggregating at the thermocline and either feeding there, or making feeding forays into the cooler near-bottom waters.

Mackerel movements into Brador Bay also appear to be inversely correlated with cod movements. Along this coast, cod have been shown to migrate inshore/offshore within the deeper cooler subthermocline water masses which upwell in response to alongshore wind forcing (Rose and Leggett 1988c). Our results indicate that inshore/offshore mackerel movements occur in association with advections of the warmer surface waters. These inverse processes are consistent with Gulf north shore fishermen's lore (G. Labadie, Lourdes-de-Blanc-Sablon, Que., pers. comm.) that when the mackerel come ashore the cod will soon move away to deeper waters.

Our data also indicate that mackerel are eurythermal and can and do tolerate water much colder than 7°C. Mackerel occurred at temperatures ranging from 0 to 13°C, with the largest densities occurring near bottom at 4–5°C. We also observed that mackerel were located in cold water even when much warmer water ($\geq 7^\circ\text{C}$) was present near the surface. These findings on temperature tolerance may reflect mackerel thermal behavior in

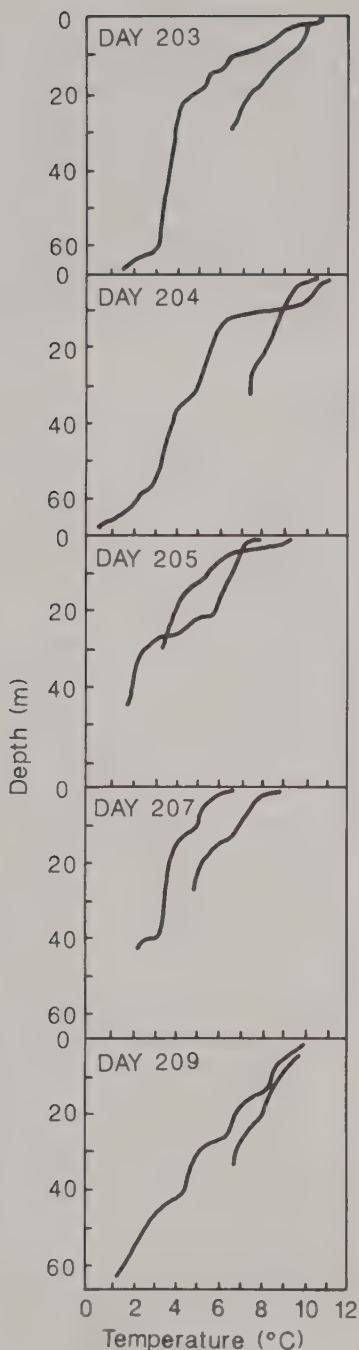


FIG. 6. Expendable bathythermograph profiles before, during, and after a major sea temperature decline at Brador Bay in 1985. The 28-m profile represents a single site; the deeper profiles represent several sites within a circular zone with radius 1.5 km.

other areas. Scott (1982) reported that this species was commonly encountered on the Scotian Shelf at temperatures ranging from 2.7 to 10°C. Giedz (1988) found that the largest catch rates by factory trawlers fishing on the U.S. mid-Atlantic Bight occurred from 5.5 to 6.5°C. Moreover, mackerel schools have been observed in water as cold as 2.9°C during their pre-

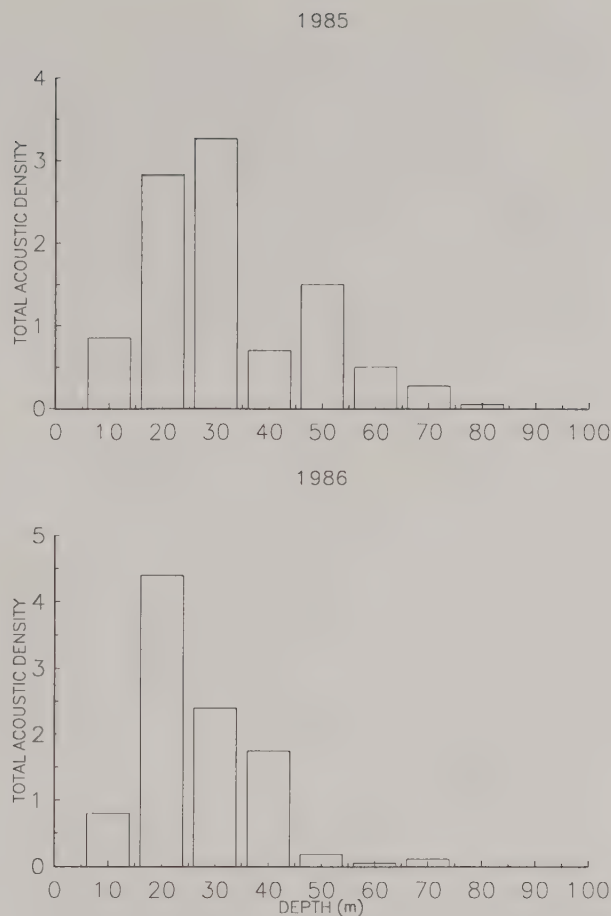


FIG. 7. Depth distribution of mackerel relative acoustic densities (V^2) in 1985 and in 1986. Each bar represents the means of a 10-m stratum (10 means from 5.0 to 14.9 m, 20 means from 15.0 to 24.9 m, etc.).

spawning migration through Cabot Strait (Gulf of St. Lawrence) (D'Amours and Castonguay 1992).

It is possible that our data on mackerel distribution relative to temperature were biased towards cooler temperatures because of vertical avoidance of the boat. Mackerel were observed to exhibit extreme lateral, near-surface avoidance of the oncoming vessel. Fish that avoided the boat in this manner were, in most cases, not ensounded. We are not aware of any specific studies of boat avoidance in this species, nor could vertical avoidance of the boat be observed in this study. However, there is evidence that Atlantic herring (*Clupea harengus*) and cod avoid oncoming vessels by descending to greater depths (Olsen et al. 1983; Misund 1990).

If, in reality, vertical avoidance of the boat was not a major factor in determining depths at which fish were recorded, it is possible that mackerel were foraging in cooler near-bottom waters during daytime hours (all acoustic observations were made in daytime) on zooplankton whose diel vertical migrations would result in daytime near-bottom aggregations. Walsh et al. (1982), who conducted a nearshore acoustic survey for mackerel off Scotland in August, also reported near-bottom distributions of mackerel schools. Invertebrate zooplankton, particularly copepods, dominate the diet of mackerel (Sette 1950; Grégoire and Castonguay 1989). Neill and Magnuson (1974) and Rose and Leggett (1989a) have reported that bluegill

(*Lepomis macrochirus*), yellow perch (*Perca flavescens*), and cod select temperatures outside their preferenda when food is more abundant there. Tanaka et al. (1987) observed that red sea bream (*Pagrus major*) feeds heavily on copepods swarming near the bottom during the daytime. In this study, mackerel occurred more frequently in cold bottom waters that were overlain by warm surface waters. This may occur because mackerel prefer to migrate vertically into warm surface waters at night when feeding ceases.

Mackerel were more likely to occur, and were more densely aggregated (as shown by the shift to higher values of mean densities compared to total densities), in nearshore waters having temperatures $\geq 7^{\circ}\text{C}$. However, mackerel were also commonly located in cold near-bottom waters when NST $\geq 7^{\circ}\text{C}$. This indicates that the fish may prefer temperatures of $\geq 7^{\circ}\text{C}$, but only if specific depth and/or off bottom distance requirements can be met.

First springtime commercial catches of mackerel have been reported to be closely associated with the appearance of the 7°C sea surface isotherm (Sette 1950; Jackman and Steven 1955; Ware and Lambert 1985). We found that mackerel are much more likely to occur and to be more densely aggregated, and hence to be much more available to fishermen, when sea temperatures are $\geq 7^{\circ}\text{C}$. However, it does not necessarily follow that the 7°C sea surface isotherm forms a barrier to horizontal and vertical movements of this species. Furthermore, the greater resolution of our observations, in both horizontal and vertical planes, relative to those of Sette (1950), Jackman and Steven (1955), and Ware and Lambert (1985) could account for the greater range of temperatures observed to be occupied by mackerel in this study.

The association of mackerel with advections of warmed surface waters indicates that this species regulates its horizontal distribution to remain within a certain, presumably optimal, range of near-surface temperatures. Hence, we hypothesize that, as has been proposed for tunas (Neill 1984), behavioral enviroregulation is a primary mechanism by which mackerel regulate their horizontal distributions during their feeding migrations. In contrast, D'Amours and Castonguay (1992) found that the timing of mackerel prespawning migration was not greatly influenced by water temperature at the entrance of the Gulf of St. Lawrence. They argued that the reproductive requirements of mackerel may override their thermal preferences. As emphasized by Reynolds (1977), successful reproduction within limits imposed by the reproductive physiology is more important for evolution than the immediate comfort of the organism. We suggest that enviroregulation may represent a major migration mechanism used by marine fishes during feeding migrations, while they may rely more on other mechanisms to achieve their prespawning migrations.

In conclusion, this study has demonstrated that mackerel movements in the nearshore zone during the feeding migration are closely linked to the wind-forced advections of heated ($\geq 7^{\circ}\text{C}$) near-surface waters but that mackerel can nevertheless be found in waters much colder than the much hypothesized 7°C boundary. It is probable that the availability of this species to the inshore fisheries could be predicted at the short time scales (<1 wk) of variations in winds and sea thermal conditions. Hence, a simple "rule of thumb" air-sea model, such as employed to guide inshore cod fishermen (Rose and Leggett)³, could be used to advantage by mackerel fishermen.

³G. A. Rose and W. C. Leggett. The use of oceanographic forecasts and echosounders to guide and enhance an inshore gillnet fishery for Atlantic cod (*Gadus morhua*). Paper presented at the International Symposium on Operational Fisheries Oceanography, St. John's, Nfld., October 23–27, 1989.

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References

- BURCZYNSKI, J. 1982. Introduction to the use of sonar systems for estimating fish biomass. FAO Fish. Tech. Pap. 191 Rev. 1: 89 p.
- CSANADY, G. T. 1982. Circulation in the coastal ocean. Reidel, Holland. 279 p.
- D'AMOURS, D., AND M. CASTONGUAY. 1992. Spring migration of Atlantic mackerel, *Scomber scombrus*, in relation to water temperature through Cabot Strait (Gulf of St. Lawrence). Environ. Biol. Fishes 34: 393–399.
- GIEDZ, M. 1988. Investigations into the relationship between shelf bottom temperature and the mackerel C.P.U.E. on the U.S. Atlantic shelf in years 1985–1987. ICES CM 1988/H:69: 14 p.
- GODIN, G. 1972. The analysis of tides. University of Toronto Press, Downsview, Ont. 264 p.
- GRÉGOIRE, F., AND M. CASTONGUAY. 1989. L'alimentation du maquereau bleu (*Scomber scombrus*) dans le golfe du Saint-Laurent et sur le plateau néo-écossais, avec une application du test de Mantel. Can. Tech. Rep. Fish. Aquat. Sci. 1673: vi + 23 p.
- JACKMAN, L. A. J., AND G. A. STEVEN. 1955. Temperature and mackerel movements in the inshore waters of Torbay, Devonshire. J. Cons. int. Explor. Mer 21: 65–71.
- LINQUIST, A., AND L. HANNERZ. 1974. Migrations of the mackerel in the Northern North Sea and in the Skagerrak. J. Cons. int. Explor. Mer 35: 276–280.
- MISUND, O. A. 1990. Sonar observations of schooling herring: school dimensions, swimming behaviour, and avoidance of vessel and purse seine. Rapp. P.-v. Réun. Cons. int. Explor. Mer 189: 135–146.
- MITSON, R. 1983. Fisheries sonar. Fishing News Books, London. 287 p.
- MOORES, J. A., G. H. WINTERS, AND L. S. PARSONS. 1975. Migrations and biological characteristics of Atlantic mackerel (*Scomber scombrus*) occurring in Newfoundland waters. J. Fish. Res. Board Can. 32: 1347–1357.
- NEILL, W. H. 1984. Behavioral enviroregulation's role in fish migration, p. 61–66. In J. D. McCleave, G. P. Arnold, J. J. Dodson, and W. H. Neill [ed.] Mechanisms of migration in fishes. Plenum Press, New York, NY.
- NEILL, W. H., AND J. J. MAGNUSON. 1974. Distributional ecology and behavioral thermoregulation of fishes in relation to heated effluent from a power plant at Lake Monoca, Wisconsin. Trans. Am. Fish. Soc. 103: 663–710.
- OLLA, B. L., A. L. STUDHOLME, A. J. BEJDA, C. SAMET, AND A. D. MARTIN. 1975. The effect of temperature on the behavior of marine fishes, p. 299–308. In Combined effects of radioactive, chemical and thermal releases to the environment. International Atomic Energy Agency, Vienna, Austria.
- OLSEN, K., J. ANGELL, F. PETERSEN, AND A. LOVIK. 1983. Observed fish reactions to a surveying vessel with special reference to herring, cod, capelin and polar cod, p. 131–138. In O. Nakken and S. C. Venema [ed.] Symposium on fisheries acoustics, selected papers. ICES/FAO Fish. Rep. No. 300, 1982, Bergen, Norway.
- OUELLET, P. 1987. Mackerel (*Scomber scombrus*) egg abundance in the southern Gulf of St. Lawrence from 1979 to 1986, and the use of the estimate for stock assessment. CAFSAC Res. Doc. 87/62: 40 p.
- OVERHOLTZ, W. J., R. S. ARMSTRONG, D. G. MOUNTAIN, AND M. TERCERIO. 1991. Factors influencing spring distribution, availability, and recreational catch of Atlantic mackerel (*Scomber scombrus*) in the middle Atlantic and southern New England regions. NOAA Tech. Mem. NMFS-F/NEC-85.
- PARSONS, L. S. 1970. Northern range extension of the Atlantic mackerel, *Scomber scombrus*, to Black Island, Labrador. J. Fish. Res. Board Can. 27: 610–613.
- REYNOLDS, W. W. 1977. Temperature as a proximate factor in orientation behavior. J. Fish. Res. Board Can. 34: 734–739.
- ROSE, G. A., AND W. C. LEGGETT. 1988a. Hydroacoustic signal classification of fish schools by species. Can. J. Fish. Aquat. Sci. 45: 597–604.
- 1988b. Atmosphere-ocean coupling in the northern Gulf of St. Lawrence: frequency-dependent wind-forced variations in nearshore sea temperatures and currents. Can. J. Fish. Aquat. Sci. 45: 1222–1233.

- 1988c. Atmosphere-ocean coupling and Atlantic cod migrations: effects of wind-forced variations in sea temperatures and currents on near-shore distributions and catch rates of *Gadus morhua*. *Can. J. Fish. Aquat. Sci.* 45: 1234-1243.
- 1989a. Interactive effects of geophysically-forced sea temperatures and prey abundance on mesoscale coastal distributions of a marine predator, Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* 46: 1904-1913.
- 1989b. Predicting variability in catch-per-effort in Atlantic cod, *Gadus morhua*, trap and gillnet fisheries. *J. Fish Biol.* 35(Suppl. A): 155-161.
- SCOTT, J. S. 1982. Depth, temperature and salinity preferences of common fishes of the Scotian Shelf, J. Northwest. Atl. Fish. Sci. 3: 29-39.
- SETTE, O. E. 1943. Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part 1. Early life history, including the growth, drift, and mortality of the egg and larval populations. U.S. Fish Wildl. Serv. Fish. Bull. 50: 149-237.
1950. Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part 2. Migrations and habits. U.S. Fish Wildl. Serv. Fish. Bull. 51: 251-358.
- TANAKA, M., H. UEDA, M. AZETA, AND H. SUDO. 1987. Significance of near-bottom aggregations as food resources for the juvenile red sea bream in Shijiki Bay. *Bull. Jpn. Soc. Sci. Fish.* 53: 1545-1552.
- VIGEANT, G. 1984. Cartes climatologiques du Saint-Laurent (fleuve et golfe). Environnement Canada, Service de l'environnement atmosphérique. 15 p.
- WALSH, M., D. N. MACLENNAN, AND J. I. EDWARDS. 1982. Results of acoustic surveys for mackerel north and west of Scotland in 1980 and 1981. ICES CM 1982/H:49.
- WARE, D. M., AND T. C. LAMBERT. 1985. Early life history of Atlantic mackerel (*Scomber scombrus*) in the southern Gulf of St. Lawrence. *Can. J. Fish. Aquat. Sci.* 42: 577-592.

Growth Comparison between Juvenile Atlantic Mackerel (*Scomber scombrus*) from the Two Spawning Groups of the Northwest Atlantic

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We compared daily growth patterns of otoliths of juvenile Atlantic mackerel (*Scomber scombrus*) younger than 91 d between the two spawning groups that occur in the Northwest Atlantic using a Gompertz model to determine whether it is possible to differentiate these groups on the basis of otolith microstructure in a mixed fishery. The growth curve parameters for the northern and southern groups, respectively, were 36.2 versus 39.7 d for the inflection point (t_0), 0.047 versus 0.040 for the instantaneous growth rate (k) when $t = t_0$, and 169.1 versus 192.5 mm for the asymptotic length at the end of the first summer of growth (L_∞). These growth curves do not significantly differ (likelihood ratio test, $P > 0.5$). It therefore appears that the otolith microstructure corresponding to the first summer would not allow identification of the group of origin.

Nous avons comparé au moyen du modèle de Gompertz les patrons de croissance quotidiens d'otolithes de maquereaux bleus (*Scomber scombrus*) juvéniles âgés de moins de 91 jours entre les deux groupes reproducteurs du nord-ouest de l'Atlantique dans le but de déterminer s'il est possible de différencier ces groupes sur la base de la microstructure des otolithes. Les paramètres de la courbe pour les groupes nord et sud, étaient respectivement 36,2 versus 39,7 jours pour le point d'inflexion (t_0), 0,047 versus 0,040 pour le taux de croissance instantané (k) à $t = t_0$ et 169,1 versus 192,5 mm pour la longueur asymptotique à la fin de la première saison de croissance (L_∞). Ces deux courbes de croissance ne diffèrent pas significativement (test du rapport de vraisemblance, $P > 0,5$). Il semble donc que la microstructure des otolithes correspondant à la première année ne permette pas l'identification du groupe reproducteur d'origine du maquereau du nord-ouest de l'Atlantique.

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Two major spawning groups of Atlantic mackerel (*Scomber scombrus*), referred to as "contingents" by Sette (1950), are known to occur in the Northwest Atlantic: a northern contingent (NC) spawning in the southern Gulf of St. Lawrence in June and July and a southern contingent (SC) spawning on the continental shelf between Cape Cod and Cape Hatteras in April and May (Sette 1943). The two contingents are managed as a single transboundary stock because there is an important fishery in the wintering area (eastern continental shelf) where they occur (Maguire et al. 1987). Developing a method to discriminate between the two contingents would allow a different management approach that would consider the origin of the fish coming from the winter fishery.

Various methods have been attempted to discriminate the two contingents of Northwest Atlantic mackerel, including meristic analyses (MacKay and Garside 1969), comparison of the par-

asitic fauna (Isakov 1976), and the genetic variability at six polymorphic loci (Maguire et al. 1987). Grégoire and Castonguay (1989) compared the otolith radius at the first annulus, while Castonguay et al. (1991) compared otolith shapes between the two contingents. Many of these studies found significant differences between contingents, but great overlaps in character distributions have prevented the development of a useful discrimination method.

SC mackerel are spawned approximately 1.5 mo before NC mackerel (Sette 1943). This difference in spawning dates could result in differences in growth rate patterns during the first growing season which could in turn be reflected in patterns of deposition of daily growth increments of otoliths (D'Amours et al. 1990). D'Amours (1992) demonstrated that extracting the record of juvenile mackerel growth from adult otoliths is possible. Hence, we hypothesized that the daily growth pattern of each contingent would differ, thus permitting the development of a method based on daily growth patterns of otoliths to determine origin in the mixed fishery.

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In this study, we assumed that interannual variability in daily growth patterns would be insignificant compared with growth pattern differences between NC and SC mackerel. If this were not the case, the stock identification method would be impractical, as new comparisons of growth rate patterns would be needed for every incoming year-class.

Daily growth patterns of fish have been compared between regions and/or stocks (Nishimura and Yamada 1988; Warlen 1988; Watanabe et al. 1988) using various growth models (reviewed by Moreau 1987). Gompertz and logistic growth models have usually been selected to describe larval and juvenile fish growth because of the inflection point included in such models (e.g. Weatherley 1987; Nishimura and Yamada 1988; Westgaard and Moksness 1990). D'Amours et al. (1990) used the Gompertz model to describe 0-yr mackerel growth. In this study, the daily growth patterns of NC and SC mackerel were compared with the Gompertz model.

Materials and Methods

Sampling

Juvenile SC mackerel 87–183 mm long (fork length (FL)) ($n = 152$) were captured between July 15 and August 15, 1989, with beach seines, gill nets, cast nets, and lines off Cape Cod, MA, USA. These fish were preserved by freezing or in anhydrous ethanol. The ethanol was replaced 2–7 d later upon return to the laboratory. Dr. A. W. Kendall (National Marine Fisheries Service, Seattle, WA) supplied the age-length data for SC mackerel 3–86 mm FL ($n = 112$) that were captured in June and July 1978 between Cape Cod and Cape Hatteras, NC (see Kendall and Gordon (1981) for details) and then preserved in 95% ethanol.

Larval and juvenile NC mackerel 4–143 mm FL ($n = 168$) were caught in the Gulf of St. Lawrence near Shippagan, N.B., in July and August 1988 and in August 1989; other NC mackerel 168–190 mm FL ($n = 7$) were captured in St. Georges Bay, N.S., from 1977 to 1980 (see D'Amours et al. (1990) for details). The specimens captured in Shippagan were preserved in anhydrous ethanol, while the otoliths of those captured in St. Georges Bay were extracted from fresh fish and kept dry until mounting.

Length Correction Factors

To correct for shrinkage of juvenile fish caused by the different preservation methods, 68 SC juveniles captured in 1989 were measured (FL and total length) and weighed fresh. Thirty-five of those were frozen at -20°C , while the rest were preserved in anhydrous ethanol for 4–5 mo. The fish were measured and weighed again just before otolith extraction, allowing the calculation of correction factors for each preservation method. For SC juveniles under 85 mm FL, an additional correction factor was calculated (using larger SC juveniles) to convert the preserved standard length that was available from Kendall and Gordon (1981) into preserved FL.

To assess larval shrinkage, NC larvae were photographed fresh and were then preserved in ethanol. The larvae were photographed again before otolith extraction. Their standard and total lengths, before and after preservation, were measured from the photographs using an image analysis system. Conversion factors were calculated from these measurements.

Otolith Microstructure

The daily periodicity of otolith (sagittae) increment deposition has been validated for mackerel larvae and juveniles from

the Northwest Atlantic. Migoya (1989) hatched mackerel eggs in the laboratory at three temperatures. She showed that the first increment of otoliths was formed the day of hatching or the day after, that the increments were deposited daily, and that water temperature did not influence the deposition rate. In addition, D'Amours et al. (1990) showed that otolith increments were deposited daily in juvenile captive mackerel.

The extraction, preparation, and otolith examination were carried out following procedures outlined in Campana and Neilson (1985). The otoliths (sagittae) were extracted after 2–5 mo of preservation. They were then cleaned and fixed on microscope slides with cyanoacrylate ("Krazy Glue") with the convex side facing the slide. Right and left otoliths were mounted on different slides.

Prior to the enumeration of the daily increments, the otoliths were polished with abrasives (3 and 30 μm) and then observed with a microscope linked to an image analysis system. The central part of the otolith was viewed at $1000\times$ magnification with immersion oil, while its outer part was examined at $250\times$ magnification. Each otolith was read by two persons. When there was disagreement after the first reading, the otolith was read again by the two readers until consensus was reached. The number of readings rarely exceeded three. The enumeration was made on the shorter axis, according to D'Amours et al. (1990). In addition to the number of increments, the following measures were taken and used in comparisons: nuclear radius (i.e. from the otolith centre to the first increment), twentieth increment radius, and total otolith radius in the reading axis.

The otoliths from NC and SC mackerel were read by two groups of two readers. NC readers, who were experienced and had had prior training on otoliths of known age (D'Amours et al. 1990), trained SC readers. The age determinations of 27 otoliths were then compared between the two groups of readers. Three independent counts of the daily increments of 25 SC otoliths were also made by SC readers to evaluate precision.

Growth Comparisons

We compared otolith/somatic growth relationships between contingents to determine whether comparing somatic growth between groups of fish using otolith growth is appropriate. We also assessed interannual variability in growth with NC fish aged between 35 and 58 d sampled in 1988 and 1989. Growth is linear for that age range; we therefore compared linear regressions of length–age using a homogeneity of slope test. For all these comparisons, variances were homogeneous (F_{max} test, $P > 0.05$).

The Gompertz model provided a better residual pattern to our data than the logistic model (Simard 1991). A Gompertz curve (see Ricker 1979, p. 705) was fitted to the length-at-age data of each contingent with the SAS nonlinear regression procedure (NLIN) using the Marquardt option (SAS Institute Inc. 1987). The model was

$$(1) \quad L_t = L_{\infty} e^{-e^{-k(t-t_0)}}$$

where L_t is FL (millimetres) at age t (days), L_{∞} is the asymptotic FL (millimetres) at the end of the first growing season, k is the instantaneous growth rate when $t = t_0$, and t_0 is the abscissa of the inflection point (the age of maximal growth). To yield parameter estimates with the smallest variance, a weighting factor equal to the inverse of age had to be introduced in the least squares analysis, as the variance in length was not homogeneous (Box et al. 1978).

The growth curves of NC and SC mackerel were compared using the likelihood ratio test, which is probably the best method

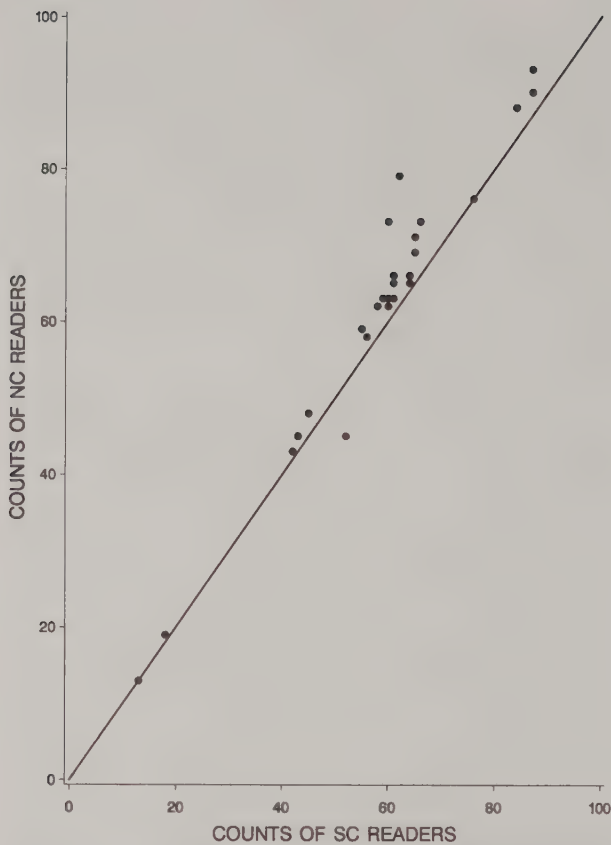


FIG. 1. Comparison of daily increment counts between the two groups of readers reading the same otoliths (NC = northern contingent; SC = southern contingent).

to compare parameters of a Gompertz model (R. M. Cerrato, Marine Science Research Center, State University of New York, Stony Brook, NY 11794-5000, USA, pers. comm.). Cerrato (1990) showed by simulation that the likelihood ratio test is the most accurate procedure among different tests (*t*-test, chi-square, Hotelling's T^2 and likelihood ratio) employed to compare von Bertalanffy parameters. Even though NC mackerel older than 90 d were available, they were excluded to standardize the age range between NC and SC mackerel.

Results

Conversion Factors

Correction factors of +2.4 and +3.7% were applied to fish preserved during 4–5 mo in ethanol and by freezing, respectively, to convert preserved FL into fresh FL. A correction factor of +3.4% was also applied to SC juveniles under 87 mm FL to convert the preserved standard length into preserved FL. For NC larvae, the preservation in ethanol caused a mean shrinkage of 5.8% of the fresh total length; lengths were corrected accordingly. For SC larvae, a correction factor of +13.0% allowed conversion of the preserved standard length into fresh total length.

The comparison of daily increment counts between the two groups of readers reading the same otoliths showed that the NC readers had systematically higher counts than the SC readers by 5.8% (Fig. 1). The ages of all SC fish were therefore

corrected accordingly. A coefficient of variation of only 3.8% was observed between different daily increment counts of the same otoliths read by the same readers.

Growth Comparison

The equations relating somatic length and otolith radius were not significantly different between the NC (eq. 2) and the SC (eq. 3) (ANCOVA, $P > 0.05$):

$$(2) \quad FL = 0.33R - 12.28 \quad df = 111; R^2 = 0.74$$

$$(3) \quad FL = 0.29R + 4.56 \quad df = 138; R^2 = 0.75$$

where FL is in millimetres, R is the otolith radius (micrometres), and R^2 is the coefficient of determination. This absence of difference indicates that a comparison of somatic growth patterns would reflect a comparison of otolith growth patterns.

The mean nuclear radius was significantly larger for NC otoliths than for SC otoliths (*t*-test, $P < 0.01$) (Table 1) whereas the mean radius at the twentieth increment was significantly larger for SC otoliths than for NC otoliths (*t*-test, $P < 0.001$) (Table 1). However, radii values overlapped greatly between the contingents, as 90 and 91% of all nuclear and twentieth increment radii values were in the overlap region, respectively.

Linear regressions relating somatic length and age were calculated for NC fish aged between 35 and 58 d captured in 1988 (eq. 4) and in 1989 (eq. 5) to evaluate interannual variability in growth:

$$(4) \quad FL = 0.82t + 50.16 \quad df = 39; R^2 = 0.14$$

$$(5) \quad FL = 3.45t - 66.92 \quad df = 38; R^2 = 0.77.$$

A significant slope difference was detected between the above regressions (homogeneity of slopes test, $P < 0.001$). The low R^2 of the 1988 relationship compared with 1989 also points to a between-year difference in growth patterns of the age range 35–58 d.

The Gompertz model provided a good description of the length–age relationship of each contingent (Fig. 2 and 3): the NC curve R^2 was 0.9849 and the SC curve R^2 was 0.9886. The maximum daily growth rate (k) was $2.92 \text{ mm} \cdot \text{d}^{-1}$ between the ages of 35.7 and 36.7 d and $2.83 \text{ mm} \cdot \text{d}^{-1}$ between the ages of 39.2 and 40.2 d for NC and SC mackerel, respectively. The average daily growth rates, calculated on 10-d intervals between the ages of 10 and 90 d, were higher for NC mackerel up to the inflection point, but then became higher for SC mackerel (Table 2).

The growth curves were not significantly different between contingents (likelihood ratio test, $P > 0.5$). The parameters of the Gompertz model, when compared one-to-one between contingents, appear to indicate different growth patterns: the 95% confidence intervals of L_∞ are separate and those of k and of t_0 overlap only slightly (Table 3). However, when the three parameters are in interaction, the resulting growth curves look much alike (Fig. 4) and are not significantly different. The greatest difference seems to lie in the asymptotic length. However, it is impossible to judge whether the L_∞ estimated by the model correspond to the real L_∞ at the end of the first growing season because no specimens older than 90 d were included in the model.

Discussion

Visual and statistical comparisons of the growth curves indicate that the two mackerel contingents exhibit similar growth

TABLE 1. Means (± 1 SD) and ranges of nuclear and twentieth increment radii of otoliths of northern and southern contingent mackerel.

	Northern			Southern		
	Mean radius (μm)	Range	<i>n</i>	Mean radius (μm)	Range	<i>n</i>
Nuclear	8.4 ± 0.8	6.6 – 10.1	97	7.7 ± 0.8	5.0 – 9.4	156
Twentieth	68.1 ± 13.9	39.7 – 96.9	112	80.2 ± 15.1	38.8 – 116.1	121

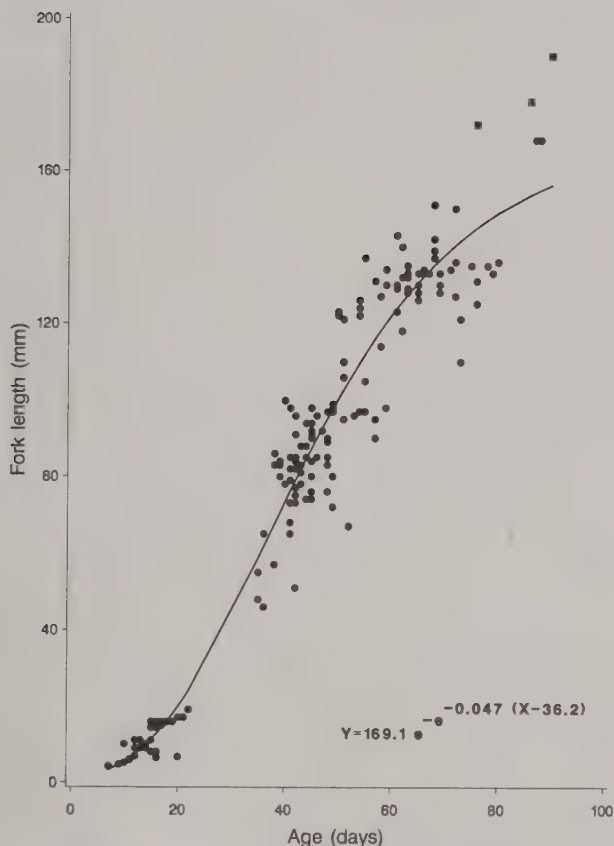


FIG. 2. Gompertz model fitted to the length-at-age data of juvenile mackerel from the northern contingent.

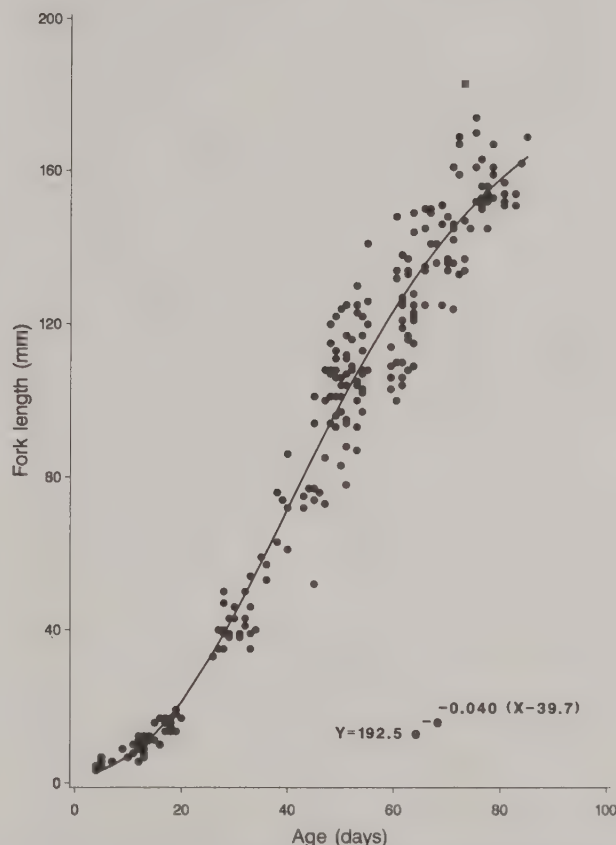


FIG. 3. Gompertz model fitted to the length-at-age data of juvenile mackerel from the southern contingent.

patterns up to 90 d old. Juvenile NC mackerel do not exhibit a higher growth rate compared with SC mackerel that would allow them to compensate for a later birth date. The slight difference in mean daily rate in favour of NC mackerel before the inflection point is offset by the larger growth rate of SC mackerel after the inflection point. It therefore appears that the otolith microstructure corresponding to the first 90 d of growth would not allow identification of the contingent of origin of adult Atlantic mackerel from the Northwest Atlantic.

The significant interannual difference in growth (between 35 and 58 d) within the NC, coupled with the absence of a growth difference between contingents when patterns from pooled years are compared, shows that our assumption on intercontingent versus interannual growth variability (see the introduction) was invalid. This constitutes another reason to conclude that the stock identification method attempted here would not be applicable.

Some dimensions of the otolith microstructure, such as nuclear and twentieth increment radii, could have allowed for

a differentiation between the two mackerel contingents. However, in spite of significant differences between the contingents, a great overlap of distributions renders those radii useless for stock identification. Similar conclusions were reached by all previous authors who attempted to develop discrimination methods for Northwest Atlantic mackerel (see the introduction). Mackerel stock separation in the Northeast Atlantic has not yet been possible either (Dawson 1991).

The asymptotic lengths (L_{∞}) yielded by the model (NC: 169 mm, SC: 192 mm) probably underestimate the actual lengths that mackerel reach by the end of the first growing season. For example, the model clearly underestimates the length of the four oldest NC mackerel (Fig. 2). D'Amours et al. (1990) also obtained a higher L_{∞} (181 mm) for NC mackerel by including fish older than 90 d. Furthermore, Grégoire and Castonguay (1989) back-calculated (using otoliths of adult fish) a mean body length at the first annulus of 207 and 223 mm for NC and SC mackerel, respectively. Differences in growth patterns might

TABLE 2. Mean daily growth rate calculated for 10-d intervals between age 10 and 90 d for the northern and southern contingents.

	Age (d)								
	10	20	30	40	50	60	70	80	90
Northern									
Fork length (mm)	5.50	19.66	44.33	73.22	100.22	121.90	137.78	148.75	156.04
Mean daily growth rate ($\text{mm}\cdot\text{d}^{-1}$)	1.44	2.45	2.89	2.70	2.17	1.59	1.10	0.73	
Mean daily growth rate between 10 and 90 d					1.88				
Southern									
Fork length (mm)	7.22	21.30	43.97	71.48	99.00	123.17	142.58	157.28	167.97
Mean daily growth rate ($\text{mm}\cdot\text{d}^{-1}$)	1.41	2.27	2.75	2.75	2.42	1.94	1.47	1.07	
Mean daily growth rate between 10 and 90 d					2.01				

TABLE 3. Estimates and 95% confidence intervals of Gompertz model parameters for the northern and southern contingents. L_{∞} = asymptotic length (mm), k = instantaneous growth rate when $t = t_0$, t_0 = inflection point of the curve (d).

	Northern		Southern	
	Estimate	95% confidence interval	Estimate	95% confidence interval
L_{∞}	169.1	157.5–180.6	192.5	181.7–203.3
k	0.047	0.042–0.052	0.040	0.037–0.043
t_0	36.2	34.2–38.2	39.7	37.8–41.6

have been found if older 0-yr fish had been included in the growth curves, as the growing season of SC fish is presumably longer. However, repeated attempts to sample older 0-yr NC and SC mackerel were unsuccessful.

The grouping of data from different years may have biased the resulting growth patterns, as we presented evidence that growth may vary among years. Comparing growth between groups of fish sampled in different years may have also biased the comparison. Grouping data from different years was necessary in order to cover the age range as much as possible. Also, had all sampling been restricted to a single year, doubts regarding the general applicability of our conclusions would have been raised. In this perspective, inclusion of data from many years may be seen as an advantage, as it provides a general description of the growth pattern of each group.

The growth patterns may have also been biased in three other ways. First, the use of various fishing methods may have biased sampling to an unknown degree. For example, slow-growing or fast-growing fish could have been sampled in higher proportions than in the population. However, it is precisely because so many gears were used (cast nets, gill nets, lines, beach seines, traps, plankton nets) that we were able to obtain a sufficient range of sizes to describe most of the first growing season. Size-selective mortality may also have biased the growth patterns. Finally, a possible underestimation of larval age by Kendall and Gordon (1981) could explain that although mean otolith radius of NC fish was larger, mean body length of 10-d-old larvae was larger for SC fish. Indeed, Kendall and Gordon's (1981) age determinations have not been validated with fish of known age.

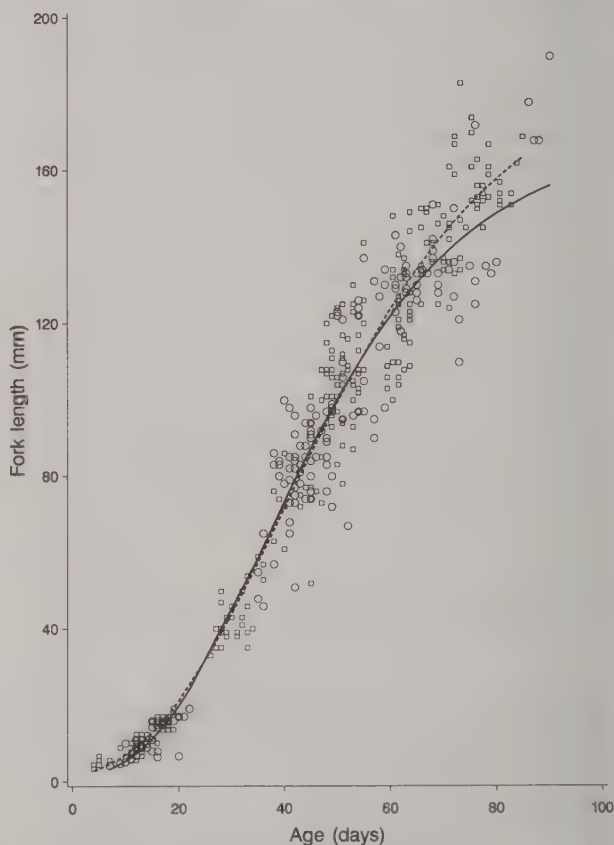


FIG. 4. Gompertz models of juvenile mackerel from the northern (solid line and circles) and southern (broken line and squares) contingents.

A slight difference in the interpretation of the deposition pattern of the increments probably explains the systematic difference in age determination by the two groups of readers. This difference was systematic, showing that there was consistency in the age determinations of each group of readers. We were unable to study in more depth this systematic difference because the microstructure of otoliths of known age had been irreme-

diably damaged by the immersion oil. Brothers (1987) reported a similar phenomenon.

Grégoire and Castonguay's (1989) back-calculations yielded a mean body length at the end of the first year only 7% shorter for NC fish than for SC fish; this is equivalent to a 19% difference in mean body weight. Assuming a peak spawning date of May 15 for the SC (Sette 1943) and of June 23 for the NC (Ouellet 1987), and assuming conservatively that growth stops as early for the SC as for the NC (October 1), it follows that the NC growing season is 28% shorter than the SC one. This comparison indicates that body length or weight of NC mackerel at the end of the first year may not be as much smaller as would be predicted solely from the shortening of its growing season. However, in the context of a crude comparison such as this one, a 19% weight difference may not be much different from a 28% shortening of the growing season. MacKay (1979) argued that mackerel lengths at the end of the first year were similar between contingents.

Conover (1990) presented evidence that in some juvenile fishes, high-latitude populations grow substantially faster than low-latitudes ones, resulting in similar sizes at the end of the first growing season across the latitudinal range (the "countergradient variation" hypothesis). Conover and Present (1990) also showed that latitudinal differences in growth rate among populations of Atlantic silverside (*Menidia menidia*) have a genetic basis. It seems unlikely that a countergradient variation mechanism could be involved in mackerel, as evidence indicates that this species has not evolved genetically divergent stocks in the Northwest Atlantic (Maguire et al. 1987) or in the Northeast Atlantic (Jamieson and Smith 1987). Moreover, because lack of genetic discreteness appears to be the rule rather than the exception in pelagic marine fishes (Jamieson and Smith 1987), the countergradient variation mechanism is probably uncommon in such fishes.

The NC spawning period, back-calculated using otoliths, is from mid-June to mid-July, agreeing with Sette's (1943) observations. Assuming a mean incubation period of 7 d (Sette 1943), we obtain a mean spawning date of June 23 for NC mackerel, which corresponds exactly to what Ouellet (1987) calculated on the basis of maximum concentrations of early-stage eggs in the plankton. By contrast, the SC spawning period back-calculated from our data (mid-May to early July) is later than what Sette (1943) reported (April and May). Our sampling at the northern end of the SC mackerel spawning area probably explains this discrepancy.

In conclusion, this study demonstrates that separation of the two spawning groups of mackerel from the Northwest Atlantic using growth rates patterns of the juvenile stage is impossible. This and previous related findings show that the current management method which considers the two contingents as a single stock is the only one possible.

Acknowledgments

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References

BOX, G. E., W. G. HUNTER, AND J. S. HUNTER. 1978. Statistics for experimenters. John Wiley and Sons, New York, NY. 653 p.

- BROTHERS, E. B. 1987. Methodological approaches to the examination of otoliths in aging studies, p. 319-330. *In* R. C. Summerfelt and G. E. Hall [ed.] Age and growth of fish. Iowa State University Press, Ames, IA.
- CAMPANA, S. E., AND J. D. NEILSON. 1985. Microstructure of fish otoliths. *Can. J. Fish. Aquat. Sci.* 42: 1014-1032.
- CASTONGUAY, M., P. SIMARD, AND P. GAGNON. 1991. Usefulness of Fourier analysis of otolith shape for Atlantic mackerel (*Scomber scombrus*) stock discrimination. *Can. J. Fish. Aquat. Sci.* 48: 296-302.
- CERRATO, R. M. 1990. Interpretable statistical tests for growth comparisons using parameters in the von Bertalanffy equation. *Can. J. Fish. Aquat. Sci.* 47: 1416-1426.
- CONOVER, D. O. 1990. The relation between capacity for growth and length of growing season: evidence for and implications of countergradient variation. *Trans. Am. Fish. Soc.* 119: 416-430.
- CONOVER, D. O., AND T. M. C. PRESENT. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. *Oecologia* 83: 316-324.
- D'AMOURS, D. 1992. A test of the adaptive value of growth for juvenile (0-year) Atlantic mackerel (*Scomber scombrus*), p. 128-132. *In* Y. de Lafontaine, T. C. Lambert, G. R. Lilly, W. D. McKone, and R. J. Miller [ed.] Juvenile stages: the missing link in fisheries research. Workshop report. *Can. Tech. Rep. Fish. Aquat. Sci.*
- D'AMOURS, D., J. G. LANDRY, AND T. C. LAMBERT. 1990. Growth of juvenile (0-group) Atlantic mackerel (*Scomber scombrus*) in the Gulf of St. Lawrence. *Can. J. Fish. Aquat. Sci.* 47: 2212-2218.
- DAWSON, W. A. 1991. Otolith measurement as a method of identifying factors affecting first-year growth and stock separation of mackerel (*Scomber scombrus* L.). *J. Cons. int. Explor. Mer* 47: 303-317.
- GRÉGOIRE, F., AND M. CASTONGUAY. 1989. Étude de dimensions au premier annulus d'otolithes de maquereau bleu (*Scomber scombrus*) du nord-ouest de l'Atlantique. *Can. Tech. Rep. Fish. Aquat. Sci.* 1680: vi + 15 p.
- ISAKOV, V. I. 1976. On some results of biological studies on mackerel from Northwest Atlantic. *ICNAF Res. Doc.* 76/52: 14 p.
- JAMIESON, A., AND P. J. SMITH. 1987. Atlantic mackerel (*Scomber scombrus*) stocks and genes: a review. *J. Cons. int. Explor. Mer* 44: 66-72.
- KENDALL, A. W., AND D. GORDON. 1981. Growth rate of Atlantic mackerel (*Scomber scombrus*) larvae in the middle Atlantic Bight. *Rapp. P.-v. Réun. Cons. int. Explor. Mer* 178: 337-341.
- MACKAY, K. T. 1979. Synopsis of biological data of northern population Atlantic mackerel (*Scomber scombrus*). *Fish. Mar. Serv. Tech. Rep.* 885: vi + 26 p.
- MACKAY, K. T., AND E. T. GARSIDE. 1969. Meristic analysis of Atlantic mackerel, *Scomber scombrus*, from the North American coastal population. *J. Fish. Res. Board Can.* 26: 2537-2540.
- MAGUIRE, J.-J., Y. C. CHAGNON, M. CASTONGUAY, AND B. MERCILLE. 1987. A review of mackerel management areas in the Northwest Atlantic. *CAFSAC Res. Doc.* 87/71: 31 p.
- MIGOYA, M. C. 1989. Étude de la croissance des larves de maquereau bleu de l'Atlantique (*Scomber scombrus*), à partir de l'examen de la microstructure des otolithes. M.Sc. thesis, Université du Québec à Rimouski, Rimouski (Québec). 90 p.
- MOREAU, J. 1987. Mathematical and biological expression of growth in fishes: recent trends and further developments, p. 81-113. *In* R. C. Summerfelt and G. E. Hall [ed.] The age and growth of fish. Iowa State University Press, Ames, IA.
- NISHIMURA, A., AND J. YAMADA. 1988. Geographical differences in early growth of walleye pollock, *Theragra chalcogramma*, estimated by back-calculation of otolith daily growth increments. *Mar. Biol.* 97: 459-465.
- OUELLET, P. 1987. Mackerel (*Scomber scombrus*) egg abundance in the southern Gulf of St. Lawrence from 1979 to 1986, and the use of the estimate for stock assessment. *CAFSAC Res. Doc.* 87/62: 40 p.
- RICKER, W. E. 1979. Growth rates and models, p. 677-743. *In* W. S. Hoar, D. J. Randall, and J. R. Brett [ed.] Fish physiology. Vol. VIII. Academic Press, New York, NY.
- SAS INSTITUTE INC. 1987. SAS user's guide: statistics, version 5. SAS Institute Inc., Cary, NC. 956 p.
- SETTE, O. E. 1943. Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part 1. Early life history, including the growth, drift, and mortality of egg and larval populations. *U.S. Fish Wildl. Serv. Fish. Bull.* 50: 149-237.
1950. Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part 2. Migrations and habits. *U.S. Fish Wildl. Serv. Fish. Bull.* 51: 251-358.
- SIMARD, P. 1991. Comparaison de la première saison de croissance entre les deux groupes reproducteurs de maquereau bleu (*Scomber scombrus*) du nord-ouest de l'Atlantique. M.Sc. thesis, Université du Québec à Trois-Rivières, Trois-Rivières (Québec). 59 p.

- WARLEN, S. M. 1988. Age and growth of larval gulf menhaden, *Brevoortia patronus*, in the northern Gulf of Mexico. Fish. Bull. U.S. 86: 77-90.
- WATANABE, Y., J. L. BUTLER, AND T. MORI. 1988. Growth of Pacific saury, *Cololabis saira*, in the northeastern and northwestern Pacific Ocean. Fish. Bull. U.S. 86: 489-498.
- WEATHERLEY, N. S. 1987. The diet and growth of 0-group dace, *Leuciscus leuciscus* (L.), and roach, *Rutilus rutilus* (L.), in a lowland river. J. Fish Biol. 30: 237-247.
- WESPESTAD, V. G., AND E. MOKSNESS. 1990. Observations on growth and survival during the early life history of pacific herring *Clupea clupea pallasii* from Bristo Bay, Alaska, in a marine mesocosm. Fish. Bull. U.S. 88: 191-200.

Feeding and Trophic Interactions of White Perch (*Morone americana*) in the Bay of Quinte, Lake Ontario¹

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Hurley, D. A. 1992. Feeding and trophic interactions of white perch (*Morone americana*) in the Bay of Quinte, Lake Ontario. Can. J. Fish. Aquat. Sci. 49: 2249–2259.

White perch (*Morone americana*) biomass declined dramatically in the Bay of Quinte in 1978 after two abnormally cold winters in 1976–77 and 1977–78. White perch diet was qualitatively and quantitatively different before and after the population decline. Changes in production and standing stocks of diet items were related to reduced eutrophy when phosphorus inputs to the bay were restricted in early 1978. The hypothesis that phytoplankton biomass in the shallow, highly eutrophic upper bay was related to white perch biomass through the benthic food web was not supported by the calculated daily consumption of benthos. White perch consumed about 5% of the daily production of chironomids in 1972–77 and less than 1% in 1978–88. In the less eutrophic, deep lower bay, predation on the amphipod *Pontoporeia hoyi* in August–September was 112% of daily production before 1978 and affected their biomass. After 1978 when white perch biomass was reduced in the lower bay, daily consumption of *P. hoyi* was 1% of daily production, and numbers of *P. hoyi* expanded greatly. Data linking consumption rate of specific diet items to production are necessary to establish trophic relationships and follow the effects of biomass shifts.

La biomasse du baret (*Morone americana*) a décliné de manière spectaculaire dans la baie de Quinte en 1978 après deux hivers anormalement rigoureux en 1976–1977 et en 1977–1978. Le régime alimentaire du baret était qualitativement et quantitativement différent avant et après le déclin de la population. Les changements dans la production et le stock actuel des composants du régime alimentaire étaient reliés à une eutrophie réduite lorsque les apports de phosphore dans la baie ont été restreints au début de 1978. L'hypothèse d'une corrélation entre la biomasse du phytoplancton dans la partie supérieure, peu profonde et hautement eutrophe de la baie, et la biomasse du baret par l'intermédiaire de la chaîne trophique benthique, n'a pas été corroborée par la consommation quotidienne de benthos calculée. Le baret a consommé environ 5 % de la production quotidienne de chironomes entre 1972 et 1977 et moins de 1 % entre 1978 et 1988. Dans la partie inférieure de la baie, profonde et moins eutrophe, la prédation de l'amphipode *Pontoporeia hoyi* entre août et septembre était de 112 % de la production quotidienne avant 1978 et affectait la biomasse. Après 1978, lorsque la biomasse du baret a été réduite dans la partie inférieure de la baie, la consommation quotidienne de *P. hoyi* est passée à 1 % de la production quotidienne et la population de *P. hoyi* a grandement augmenté. Les données mettant en relation le taux de consommation d'éléments particuliers du régime alimentaire et la production sont nécessaires pour établir les relations trophiques et suivre les effets des changements au niveau de la biomasse.

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The purpose of this study is to examine the trophic interactions between white perch (*Morone americana*) and their prey in two ecologically distinct areas of the Bay of Quinte to explain the links between white perch biomass, their prey, and phytoplankton biomass. Nicholls and Hurley (1989) showed that over 50% of the variance in a multiple regression model linking phytoplankton biomass with five input variables was explained by the biomass of white perch. We postulated that one possible connecting link between these two factors was the biomass of macrobenthos.

In the shallow upper Bay of Quinte, benthic chironomids, particularly *Chironomus plumosus* f. *semireductus*, use the filamentous diatom *Melosira* as an important diet item (Johannsson and Beaver 1983). *Melosira*, along with blue-green algae, dominate the phytoplankton and may impede the development of large *Daphnia* populations (Nicholls and Hurley 1989).

There is no significant correlation between the abundance of *Daphnia galeata mendotae* and chlorophyll *a* concentrations in the upper bay (Strus and Hurley 1992), probably because *D. galeata mendotae* do not readily utilize long filamentous phytoplankton. For these reasons, it appears that the link between macrobenthos and phytoplankton may be more direct than the one usually postulated (McQueen et al. 1986) between phytoplankton and zooplankton.

White perch prey heavily upon benthic macroinvertebrates, particularly chironomids, in the shallow upper Bay of Quinte. It is through this link to the filamentous phytoplankton that white perch abundance was directly related to phytoplankton biomass: the so-called "benthic-link" hypothesis (Nicholls and Hurley 1989).

A major reduction in white perch abundance occurred in the late 1970s precipitated by two successive abnormally cold winters (Ridgway et al. 1990; Johnson and Evans 1990). While white perch numbers have increased since the reduction, their numbers are still generally significantly below the early 1970s values.

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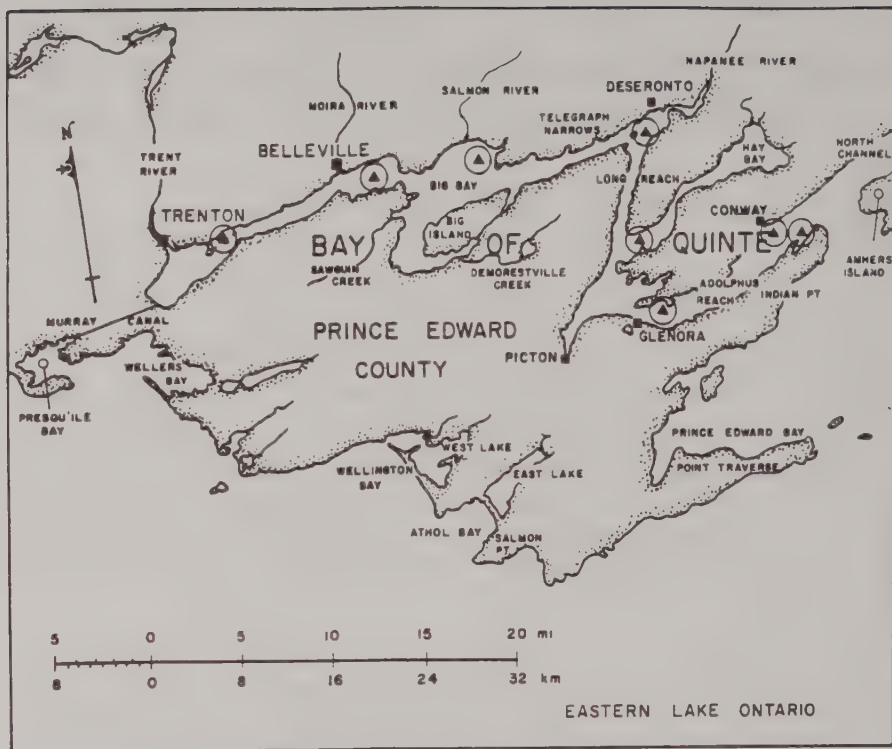


FIG. 1. Locations (solid triangles) of bottom trawling sites in the Bay of Quinte, Lake Ontario.

Shifts in the standing stocks of prey of white perch occurred in the 1970s and 1980s when phosphate control programs were established in the bay. Phytoplankton biomass dropped significantly for a few years after phosphate control began but then increased sharply by 1984–85 (Nicholls and Hurley 1989). Although the species composition among macrozooplankton remained relatively unchanged, there were significant increases in the daily net production of several species of cladocerans and copepods after 1976 (Cooley et al. 1986). Among the benthic macroinvertebrates, the daily net production and biomass of chironomids decreased in the shallow upper bay with decreased phosphorus loads, while they increased in the deep lower bay for chironomids and amphipods (Johnson and McNeil 1986).

The effect of changing white perch abundance on the consumption of their prey was examined by calculating both daily consumption and net production in both the shallow upper bay and the deep lower bay over a period of 15 yr. Johnson and McNeil (1986) postulated that white perch in the lower bay were a significant factor in restricting the expansion of the amphipod *Pontoporeia hoyi* to other suitable habitats in that area. I tested this "predation" hypothesis by calculating the consumption of macroinvertebrates by white perch over a period of years when their abundance shifted significantly. Similarly, the "benthic-link" hypothesis was tested by calculating the consumption of chironomids by white perch in the upper bay when the abundance of white perch changed significantly. The extent of the impact on net production of these benthic species by white perch predation would form the basis of rejection of either or both of these hypotheses.

Methods

Sampling Location and Fishing Gear

From 1972 to 1980, four sites were fished using a bottom trawl in the upper Bay of Quinte between Trenton and Deseronto (Fig. 1). Catches from these sites were pooled to form the upper bay series. The annual number of trawl tows ranged between 15 and 20. Three sites were fished in the lower Bay of Quinte from Glenora to Indian Point (Fig. 1) during the same time period and pooled as the lower bay series. The annual number of trawl tows ranged from 8 to 15. From 1981 to 1988, only one site was fished in the upper bay (Big Bay) and the annual number of trawl tows ranged from 1 in 1982 to 12 in 1988. Similarly, at the single site in the lower bay (Conway) the annual number of trawl tows ranged from 1 to 12.

A 3/4 Western bottom trawl was used each month, June–September, and dragged over a distance of 400 m at a speed of 1.1 m/s. The trawl was 19 m long with 6-m wings and 1.3-cm stretched mesh in the codend. Trawling depth in the upper bay was about 5 m and the lower bay was between 18 and 22 m.

The trawl tows were limited to areas where the bottom was relatively smooth and free of obstructions. The trawl doors and warps may have caused some degree of herding of fish; however, Blaxter et al. (1964) stated that this reaction depends upon the degree of visibility of the net. Because water transparency in the bay is relatively low with mean light extinction coefficients in the upper bay of 1.62/m and in the lower bay of 0.60/m (S. Millard, Great Lakes Laboratory for

Fisheries and Aquatic Sciences (GLLFAS), Burlington, Ont., pers. comm.), the net would be invisible at a distance of a few metres in the water.

The bottom trawl tended to capture a broader size range of white perch than did multimesh experimental gill nets set in the same location (Minns and Hurley 1986). However, both gear types recorded the dramatic reduction in white perch abundance witnessed in 1978 (Hurley 1986a) and thus corroborate major shifts in the white perch population.

Catch Processing

Each catch was removed from the trawl and sorted by species, counted and weighed, and packed in crushed ice to slow further digestion of stomach contents. If available, samples of 30 white perch were selected from each tow and the fork length and weight recorded for each fish. The sex of each fish was determined and scale samples were removed for age determination. Stomachs were removed from 6–10 of the fish and preserved in 10% buffered formalin.

Calculation of Fish Biomass

An estimate of the areal biomass was made from the fact that each tow covered 0.2453 ha. Echograms were made while the trawl was operating and indicated the concentration of fish within a few metres of the bottom. Because the trawl height was 2.5 m, the catch would include these concentrations such that areal estimates would be more meaningful than volume estimates. Biomass calculations from trawl tows in separate areas of the upper bay were combined to produce a geometric mean (N between 4 and 20) for the June–September period each year. A similar estimate was produced for the lower bay sites (N between 3 and 15). A 95% confidence interval was calculated using a formula given by Craig et al. (1986).

In order to examine seasonal effects, the fish samples were further subdivided into two summer periods, June–July and August–September. White perch biomass was examined in each of these periods in both the upper and lower bays. Biomass was partitioned into 25-mm length classes by applying the length frequency distribution obtained from the subsample to the total catch. Thus the proportion of biomass among size groups of white perch could be separately examined. This was particularly important in 1978–88 when the abundance of young-of-the-year (YOY) white perch increased significantly.

The samples were pooled for the years 1972–77, the period before the dramatic population decline, and 1978–88, the period after the decline.

Fish Stomach Analysis

Food organisms were removed from the stomachs and identified to the most specific level possible. The lengths of amphipods, isopods, sphaeriids, chironomids, and trichopterans were measured and weights were calculated from the regression equations of Johnson and Brinkhurst (1971). The weights of macrozooplankton were obtained from Hall et al. (1970) and J. Cooley (GLLFAS, pers. comm.). All weights are expressed as ash-free dry weight (AFDW) which most closely corresponds to organic weights and thus energy content (Johnson and Brinkhurst 1971).

Prey Sampling

Zooplankton

Zooplankton samples were collected weekly by GLLFAS from May to the end of September at up to eight stations in the

upper and lower bays in 1975, 1976, and 1979–88. From 1983 to 1988, samples were obtained biweekly at two sites: Belleville and Conway (Fig. 1). Seasonal mean abundances and biomasses were calculated over the sampling period.

Samples were taken with a 30-L Schindler-type sampler (Schindler 1969) with a 75- μ m mesh and bucket and were preserved in 10% buffered formalin. Samples were collected at 1-m intervals in the upper bay. In the lower bay, samples were collected at between 3- and 5-m intervals to the 30-m level. Abundances (number per cubic metre) were estimated from depth-weighted volumetric means.

The density of any species dictated the proportion that was enumerated. Generally, about one tenth of each sample from the upper bay and one quarter of each sample from the lower bay were counted. A minimum of 300 individuals was counted in any subsample. Precision of species estimates would be greatest for the most abundant forms.

Zooplankton production estimates require data on egg development in relation to water temperature (Cooley et al. 1986). Eggs of *Bosmina longirostris* and *Eubosmina coregoni* were incubated at several temperatures to produce development curves. Literature values were used for *Daphnia* spp. and are listed in Cooley et al. (1986). All cyclopoid and calanoid copepod production was derived by treating eggs as though they were from a single species. This approach was necessary primarily because of the difficulty in determining species of immature instars and also because of the lesser abundance of these forms compared with the cladocerans. Cooley et al. (1986) detail the calculations and techniques used to derive the estimates of production. Data for the years 1984–88 were obtained from the continuing sampling series in the bay reported by J. Moore (GLLFAS, pers. comm.).

I converted mean seasonal production of the major zooplankton groups to daily production by setting the season length to 134 d (May 12 – September 23) as described by Cooley et al. (1986). I converted the dry weight values to AFDW using $AFDW = 0.95 \times \text{dry weight}$ (J. Cooley, pers. comm.). Finally, multiplying the volumetric unit (cubic metres) by the mean depth (3.17 m at Belleville and 23.75 m at Conway) converted measurements to areal units (square metres).

Macrobenthos

Samples were obtained with a modified Ekman dredge at one site in the upper bay (Big Bay) and at two sites in the lower bay (Glenora and Conway) (Fig. 1) at varying yearly intervals between 1967 and 1987 (Johnson and McNeil 1986; R. Dermott, GLLFAS, pers. comm.). The most extensive sampling was done in August to ensure that YOY *P. hoyi* would be collected. All samples were screened through 0.6-mm-aperture brass screens. Six dredge samples were taken in each year at each index station. Additional stations outside the index locations were sampled at varying yearly intervals and intensity. The database is derived from approximately 800 Ekman samples. Biomass was determined by volume displacement in 70% ethanol. Appropriate conversion factors were used to express biomass in grams AFDW per square metre (Johnson 1970).

Net production of the major species was determined according to Johnson (1970) from laboratory tests to determine respiration rates and growth rates. Daily turnover times (biomass divided by daily production) were calculated from monthly sampling in the upper and lower bays from April 1967 to September 1968 (Johnson 1970). Mean daily turnover times were estimated for the June–July and August–September periods, and these expressed net production in terms of renewal time.

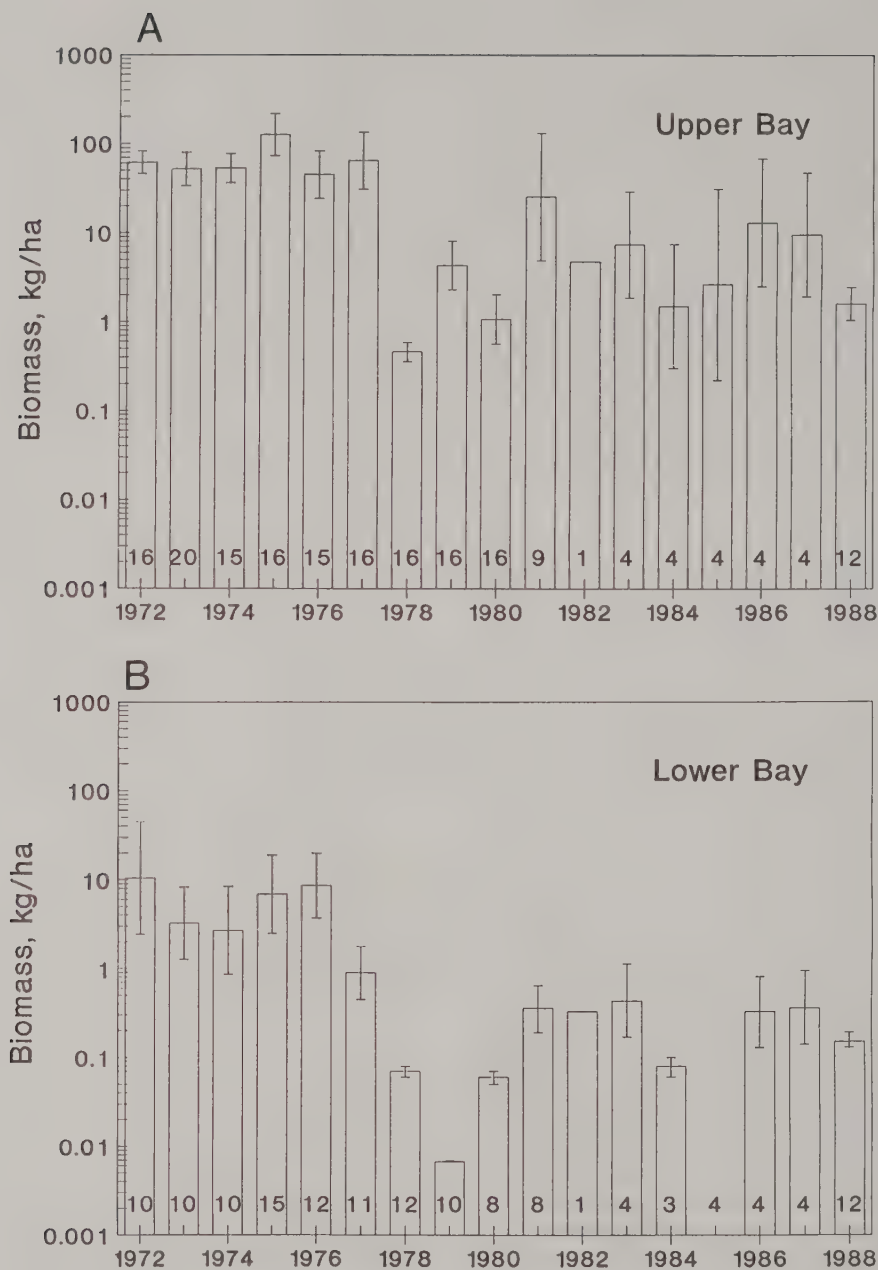


FIG. 2. White perch geometric mean June–September biomass in the (A) upper Bay of Quinte and (B) lower Bay of Quinte; 95% confidence limits and the number of replicates are shown.

Finally, using daily turnover rate, the inverse of daily turnover time, and the average biomass present for the different benthic groups, an estimate of the daily production was derived. August samples adequately reflect the mean summer biomass of macrobenthos in the upper bay but would overestimate amphipod biomass and underestimate chironomid biomass in the lower bay (Johnson 1970).

Calculation of Daily Food Consumption

An estimate of the daily food consumption (C_0) was made from the equation of Elliott and Persson (1978)

$$C_0 = 24a \sum_{i=1}^n (S_i N_i)$$

where S is the mean amount of food in the stomach over a 24-h period, a is the exponential rate of gastric evacuation, n is the ordinal number of size class, and N is the number of white perch in that size class. Mean daily food consumption was calculated for each 25-mm length class for the upper and lower bays and for the June–July samples and the August–September samples in the 1972–77 and 1978–88 periods. The equation to calculate a is $\ln(100/y)$ where y is the time in hours required

TABLE 1. Mean seasonal (May–October) abundance and biomass of major zooplankton in the upper and lower Bay of Quinte. Significance of differences by *t*-tests **P* < 0.05; ***P* < 0.01.

	Upper bay				Lower bay			
	1975–76		1979–88		1975–76		1979–88	
	No./m ²	mg AFDW/m ²	No./m ²	mg AFDW/m ²	No./m ²	mg AFDW/m ²	No./m ²	mg AFDW/m ²
Cladocera								
<i>Bosmina longirostris</i>	134288	100.7	159175	119.4	469229	351.9	1046496	784.9
<i>Eubosmina coregoni</i>	168089	126.1	264137	198.1	112931	84.7	203894	152.9
<i>Daphnia retrocurva</i>	49937	137.3	79345	218.2	51395	141.3	158674**	436.4
<i>Daphnia galeata mendotae</i>	498	1.4	15983**	44.0	974	2.7	4845	13.3
<i>Ceriodaphnia lacustris</i>	13669	37.6	25706	70.7	38618	106.2	108181*	297.5
<i>Leptodora kindtii</i>	1465	20.4	479*	6.7	1378	19.2	784	10.9
Copepoda								
<i>Diacyclops bicuspidatus thomasi</i>	393	3.3	631*	5.2	44508	369.4	82793	687.2
<i>Cyclops vernalis</i>	5475	45.4	7142	59.3	3301	27.4	2138	17.7
<i>Mesocyclops edax</i>	846	7.0	4508**	37.4	3895	32.3	6840	56.8

for 100% evacuation of the stomach contents at any specific temperature. The consumption of each diet item was estimated by prorating the total daily consumption by the proportion that each item was found in the diet.

Windell (1978) listed times to evacuation at a series of temperatures for *Perca fluviatilis*. Because white perch and yellow perch share many of the same food items and are generally similar in size and morphology (Hurley 1986b), the values listed by Windell (1978) were used here. Using data provided by Windell (1978), the equation relating time to 100% evacuation with temperature (*T*) is

$$y = -2.114 + 587.386/T \quad (R^2 = 0.99).$$

Water temperatures were available for the upper bay from the intake of the Belleville Utilities Commission water filtration plant. These daily temperatures track the temperature in the shallow upper bay from Trenton to Deseronto. Mean June–July temperature at depths of 5 m was 22.0°C (95% CL 21.3–22.6°C) and mean August–September temperature was 21.1°C (95% CL 20.2–21.9°C). For the lower bay, monthly limnological surveys were made in the area over the years of the study and temperatures were obtained at 20 m depth to approximate the region where tows were made. Mean June–July temperature was 12.4°C (95% CL 11.1–13.7°C) and mean August–September temperature was 16.5°C (95% CL 15.8–17.2°C).

Results

White Perch Biomass

White perch biomass in the upper bay ranged generally between 50 and 100 kg/ha between 1972 and 1977 but suffered a significant decline in 1978 that persisted through 1979 and 1980 (Fig. 2A). By 1981, mean biomass had increased in the upper bay but was still below the 1972–77 means. A reduction in effort after 1980 resulted in larger variances that made the confidence intervals quite large (Fig. 2A). Another less drastic reduction in white perch biomass was noted in 1984 and 1985. However, variance of the 1985 samples was so large that the 95% confidence limits included nearly all levels of abundance in other years. In 1988 when 12 samples were taken, the variance was greatly reduced and the mean biomass in that year was about one-tenth the peak biomass in 1972–77 (Fig. 2A).

White perch were never as abundant in the lower bay as they were in the upper bay (Fig. 2B). Biomass in the 1972–77 period was about 5 kg/ha. In fact, biomass in 1977 was considerably below the average of previous years. A sharp reduction occurred in 1978–80 as in the upper bay, but a partial return to previous values occurred in 1981–83. Again, biomass since 1986 was approximately one-tenth the earlier levels (Fig. 2B). Variance in the lower bay series beginning in 1978 has been much less than that recorded in the upper bay.

Prey Abundance and Biomass

Zooplankton

The mean seasonal abundance and biomass of the major zooplankton species that formed a significant portion of the white perch diets increased from the 1972–77 period to the 1978–88 period, although statistically significant increases were recorded for only some species (Table 1). The most common cladocerans were the small *B. longirostris* and *E. coregoni* (0.25–0.54 mm long). The other major species was *Chydorus sphaericus*; however, because it never formed a significant portion of the diet, it was not included in Table 1. *Ceriodaphnia lacustris* was somewhat larger (0.43–0.54 mm long) than the smaller bosminids. *Daphnia retrocurva* (0.58–0.96 mm long) numbers increased significantly in the lower bay, while *D. galeata mendotae* (0.83–1.14 mm long) increased significantly in the upper bay (Table 1). *Leptodora kindtii*, the largest cladoceran with lengths up to 17 mm, decreased in abundance in the upper bay (Table 1). This species with its prominent pigmented eye spot was heavily preyed upon by small (2–5 g) walleye (*Stizostedion vitreum*, previously *S. vitreum vitreum*) in the late 1970s and through the 1980s (Hurley 1986c) and its decline may be related to this predation.

Copepod abundance was not as high as that of the cladocerans, but copepods were somewhat larger in size than the cladocerans. Significant increases were observed for two copepod species in the upper bay (Table 1). Large numbers of cyclopoid copepodites and nauplii were recorded from both the upper and lower bays and also were part of the white perch diets, but these immature stages could not be separated by species.

Macrobenthos

Chironomids were generally more abundant in the upper bay in the 1967–77 period (500–2500/m²) than from 1982 to 1987

(100–1400/m²) (R. Dermott, pers. comm.). In the lower bay, however, chironomid abundance was more variable over the same period. Maximum numbers were recorded at one site in 1986 (3000/m²). Generally, chironomids were never as abundant in the lower bay at Conway (up to 500/m²) compared with the other stations.

Amphipods were never commonly found in the upper bay. However, amphipods (in particular *P. hoyi*, which was the major species found) were very numerous in the lower bay in several years (1977, 1982, 1986, 1987) when up to 12 000/m² were taken. This increase has been related to a possible return to optimal dissolved oxygen conditions and a reduction in predation pressure as a result of reduced white perch abundance (Johnson and McNeil 1986).

Standing stocks (grams AFDW per hectare) of chironomids declined in the 1977–84 period in the upper bay, but increased in the lower bay (Table 2). Amphipod biomass increased greatly in the 1982–84 period in the lower bay compared with 1977, while isopod biomass decreased (Table 2). Sphaeriids were not plentiful in the upper bay and only marginally so in the lower bay (Table 2).

Diet Composition and Biomass

Upper Bay of Quinte

Although white perch biomass fell sharply after 1977, the diet was still dominated by chironomids in both time periods, 1972–77 and 1978–88, and in each summer interval in the upper bay (Fig. 3). Some reduction in the mean percentage of chironomids occurred between the two time periods, from about 90 to about 70%. However, the mean biomass of stomach contents from fish in the upper bay nearly doubled after the population crash in both the June–July and August–September samples (Table 3).

In June–July the proportion of trichopterans, cladocerans, and culicids increased in 1978–88 over the earlier period (Fig. 3A), while the August–September samples showed increases in copepods and, to a lesser extent, cladocerans and trichopterans (Fig. 3B). The increased copepod consumption was the result of the large numbers of YOY white perch present. Among cladocerans, there was an increase in the biomass of large-bodied forms such as *Daphnia pulex*, *L. kindtii*, and *D. galeata mendotae* in the 1978–88 samples. In earlier years, *B. longirostris* and *E. coregoni*, both small-sized cladocerans, were the major cladocerans in the diet.

The biomass of trichopterans eaten also increased in the June–July 1978–88 series (Fig. 3A) with the predaceous *Oecetis* spp. and *Polycentropus* sp., which prey largely on chironomids (Wiggins 1977), accounting for the increase.

The August–September samples from the upper bay showed the importance of copepods in white perch diets, particularly among the YOY. *Acanthocyclops vernalis* and *Mesocyclops edax* were the major copepod species eaten.

Gammarus fasciatus was the dominant amphipod in the diet, but amphipods never formed a large proportion of the white perch diet in the upper bay. Similarly, gastropods were never significant diet items in the upper bay (Fig. 3A and 3B).

Lower Bay of Quinte

Chironomids, amphipods, isopods, and trichopterans were the major diet items of white perch from the lower bay in both summer periods and in both groups of years studied (Fig. 4). The few samples in 1978–88 make direct comparisons difficult. However, in 1972–77 the June–July series showed that the diet was fairly evenly distributed among chironomids, amphipods, and trichopterans with somewhat fewer isopods. In the August–September samples, trichopterans were a minor diet item with an increased proportion of isopods present. In 1978–88, amphipods increased greatly as diet items. The few samples in June–July showed a similar trend. *Gammarus fasciatus* and *P. hoyi* were the major amphipods eaten with *P. hoyi* increasing in importance after white perch numbers declined in 1977.

The August–September series showed the importance of isopods (*Asellus* sp.) in 1972–77 but not in the later years (Fig. 4B). Among the copepods, *M. edax* and *A. vernalis* were important diet items in 1978–88, especially among YOY white perch. Chironomids in this later period were not as prevalent in the diet as they had been previously. This may have resulted from the large numbers of YOY white perch present which did not depend as heavily upon chironomids compared with copepods.

Calculated Daily Consumption and Production of Food Items

The significant effect of the steep decline in the white perch population in 1978 is particularly well illustrated by the drop in the calculated daily consumption of chironomids in the upper bay (Table 4). In June–July the 1978–88 consumption was 95.4% less than the 1972–77 value and 92.2% less for the August–September series. Consumption of cladocerans and copepods increased in the June–July 1978–88 series when large numbers of small (25–50 mm fork length) YOY white perch were present but decreased in August–September (Table 4). The daily consumption of amphipods in the June–July samples did not change between the two time periods, but the consumption during the August–September period in 1978–88 was 86.6% less than that in the 1972–77 period. Daily consumption of trichopterans and gastropods was reduced after 1977.

In the lower bay, daily consumption of all diet items except amphipods and isopods was less than in the upper bay (Table 5). Consumption of chironomids in August–September 1978–88 was 98.4% less than the pre-1978 value. Consumption of both amphipods and isopods was greatly reduced after 1977 (92.3 and 98.9%, respectively). Copepods were the only zooplankters eaten in any significant amount and their utilization increased by 300% after 1977 (Table 5). This reflected the abundance of YOY white perch at that time.

TABLE 2. Mean standing stock (g AFDW/ha) of benthic macroinvertebrates from the upper and lower Bay of Quinte sampled in August (standard errors in parentheses). Data from Johnson and McNeil (1986).

	Upper Bay			Lower Bay		
	1967–68	1977	1982–84	1967–68	1977	1982–84
Chironomids	6400 (620)	14100 (1370)	4800 (1000)	2100 (415)	200 (90)	2900 (650)
Amphipods	200 (200)	—	167 (147)	57350 (1970)	500 (340)	13900 (2800)
Isopods	—	—	—	2400 (355)	12000 (2710)	900 (360)
Sphaeriids	<100	—	—	1900 (175)	—	—

TABLE 3. Numbers of white perch stomachs examined from upper and lower Bay of Quinte in the 1972–77 and 1978–88 periods in June–July and August–September, together with the geometric mean biomass of white perch and the mean AFDW of stomach contents per gram wet weight of white perch.

	Upper bay				Lower bay			
	1972–77		1978–88		1972–77		1978–88	
	June–July	Aug.–Sept.	June–July	Aug.–Sept.	June–July	Aug.–Sept.	June–July	Aug.–Sept.
N	257	260	154	169	127	155	7	23
Geometric mean								
Biomass (kg/ha)	48.8	79.2	2.04	4.27	2.34	10.05	0.01	0.29
(95% CL)	(36.3–65.6)	(60.2–104.1)	(1.34–3.13)	(2.82–6.46)	(1.50–3.67)	(5.27–19.2)	— ^a	(0.25–0.34)
Mean stomach								
contents (mg AFDW/g)	0.247	0.130	0.437	0.274	0.698	0.644	— ^a	0.917

^aNumbers insufficient to warrant calculation.

Daily net production was calculated for the three main benthic groups and compared with the derived daily consumption (Table 6). In the upper bay, the maximum daily consumption of chironomids was only about 5% of the daily net production before 1977, and after 1977 the proportion was less than 1%. In the lower bay, daily consumption of chironomids in 1972–77 was 38% of the net production during June–July and exceeded (168%) the daily net production in August–September (Table 6). After 1977, chironomid production increased but the proportion consumed decreased greatly. Consumption of amphipods in 1972–77 was 13% of the net production in June–July and exceeded net production (112%) in August–September. After 1977, however, net production increased greatly and consumption became less than 1% of net production (Table 6). Consumption of isopods was never a large proportion of the net production.

Daily net production of cladocerans and copepods increased in the bay after 1976 (Table 7). Cladocerans increased by 18.0% in the upper bay and by 77.7% in the lower bay. Daily net production of cyclopoid copepods increased over sevenfold in the upper bay and over threefold in the lower bay.

Daily consumption of cladocerans in the upper bay was only a small fraction of the daily net production (Tables 4 and 7). Daily consumption of cyclopoid copepods in August–September 1972–77 was 18% of the daily net production, but in 1978–88 was only 2% of the net production. Daily consumption of copepods in the lower bay was always a very small fraction of the daily net production (Tables 5 and 7).

Discussion

Two unrelated events occurred in the Bay of Quinte in the late 1970s that significantly altered the bay's trophic structure. One event was the 50–60% reduction in phosphorus inputs from sewage treatment plants that began in early 1978. The other was the occurrence of two successive abnormally cold winters in 1977 and 1978 that resulted in major declines in alewife (*Alosa pseudoharengus*) and white perch (Hurley 1986a; Johnson and Evans 1990; Ridgway et al. 1990). In addition, significantly large year classes of yellow perch and walleye developed in the spring of 1978 (Hurley 1986a). Speculation suggests that walleye resurgence was related to the white perch decline (Hurley and Christie 1977; Hurley 1986a).

The reduction in white perch numbers after 1977 resulted in a significant increase in growth as indicated by their length at age (Hurley 1986a). Sheri (1968) also reported that white perch growth in the Bay of Quinte was inversely related to abundance. Additional studies on the age structure of white perch in the

bay confirmed that upper bay fish at every age were significantly larger than lower bay fish in the 1972–87 period discussed here (D. A. Hurley, unpubl. data). Furthermore, fish captured in both areas of the bay after 1977 were significantly larger than those captured before then (D. A. Hurley, unpubl. data). Only among YOY white perch in 1978–87 in the upper bay was growth less than in 1972–77. This was probably the result of the very large numbers of YOY white perch present in those years (Hurley 1986a). Minns and Hurley (1986) described a model in which the growth of YOY white perch was directly related to water temperature and inversely related to their numbers.

The mean weight of white perch stomach contents per unit of fish weight increased in both the upper and lower bay after 1977 (Table 3). This would suggest greater growth associated with greater food consumption. In addition, the proportion of net production of most diet items consumed by white perch decreased markedly after 1977 which suggests that food was not a limiting factor for growth.

With the reduction in phosphorus loadings, there was a similar percentage drop in phytoplankton biomass for a few years (Nicholls et al. 1986). Macrozooplankton in the bay did not change significantly in the first years following phosphorus control; small-bodied cladocerans dominated the biomass (Cooley et al. 1986). In later years, after 1981, both biomass and production of cladocerans and, to a lesser extent, copepods increased, particularly in the upper bay (J. Moore, pers. comm.). *Daphnia galeata mendotae* abundance has gradually increased since 1982 and represents a shift to larger cladocerans. The numbers and biomass of benthic macroinvertebrates declined between 1977 and 1984, probably as a consequence of lower phosphorus inputs (Johnson and McNeil 1986). Since 1984, however, the numbers of amphipods and, to a lesser extent, isopods have increased substantially in the lower bay.

The hypothesis that phytoplankton biomass, at least in the upper bay, is controlled by chironomids that feed on the dominant filamentous diatom *Melosira* (Johannsson and Beaver 1983) and that chironomid biomass is controlled by white perch predation (Nicholls and Hurley 1989) was tested by determining the consumption of chironomids by white perch. When white perch abundance was high in years before 1978, much of the biomass and production of chironomids, if the hypothesis is correct, should have been consumed by white perch. In fact the data show that in 1972–77, chironomid consumption by white perch was not greater than 5% of the daily net production of chironomids. After 1977 when the standing stock and production of chironomids in the upper bay were reduced, less than

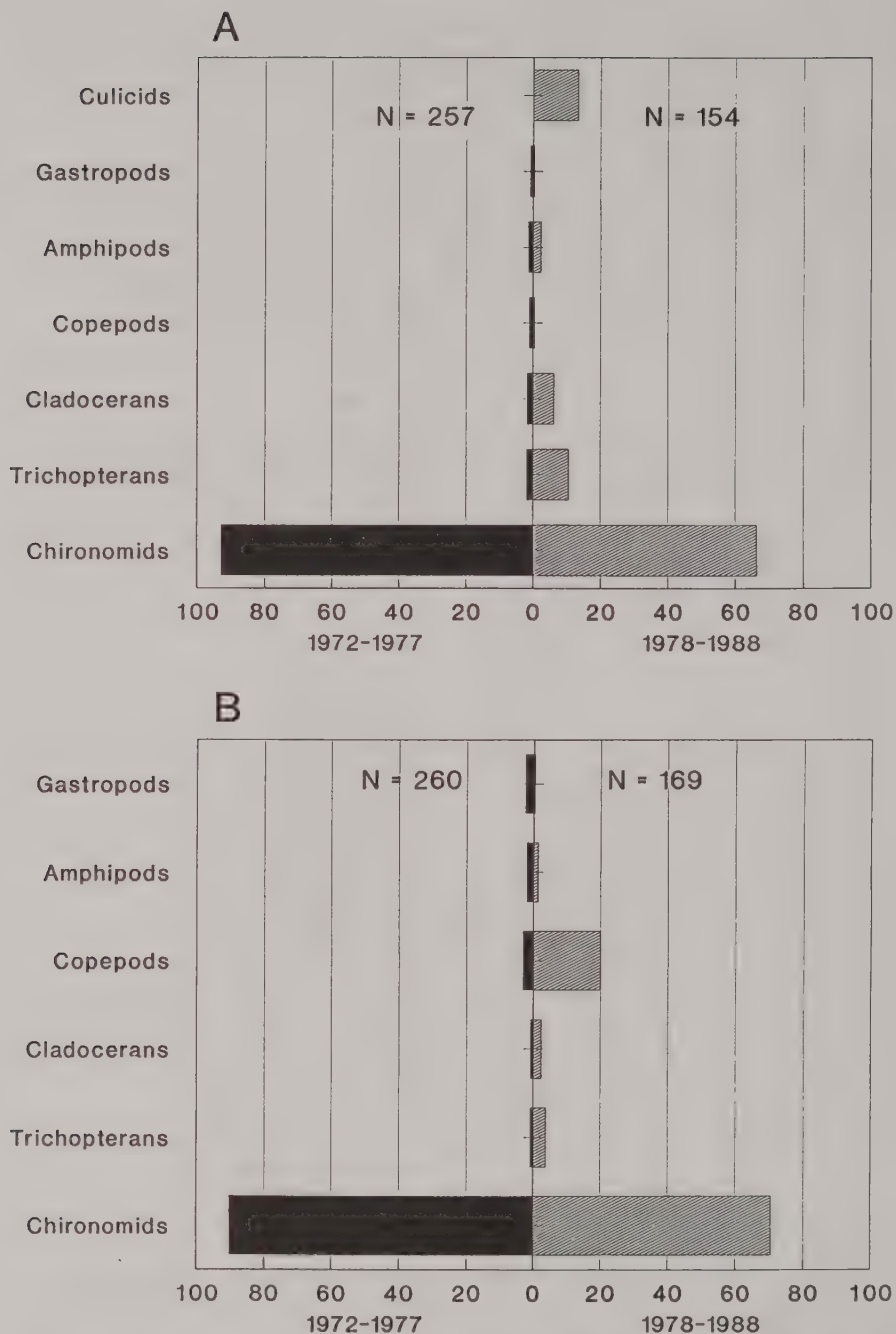


FIG. 3. Percent composition of white perch diet biomass by major taxa in 1972-77 (solid bars) and 1978-88 (striped bars) in the upper Bay of Quinte in (A) June-July and (B) August-September.

1% of the daily net production was consumed. The data, therefore, apparently fail to support the "benthic-link" hypothesis.

Nicholls and Hurley (1989) examined a suite of environmental variables plus total phosphorus and total nitrogen concentration, in addition to the mean summer biomass of white perch and alewife in a stepwise regression with mean May-October phytoplankton biomass as the dependent variable. While the stepwise technique excluded some variables, examination of the correlation matrix for these variables showed sig-

nificant correlations between some of them. As a further test of the significance of these variables, we did a principal components analysis using all the independent variables and found that the first four components were significant and these included both white perch and alewife biomass. It is possible that the white perch biomass may have acted as a surrogate for other benthic feeding fishes such as yellow perch and brown bullhead (*Ameiurus nebulosus*, previously *Ictalurus nebulosus*) (Hurley 1986b). The percentage of the total diet of the main

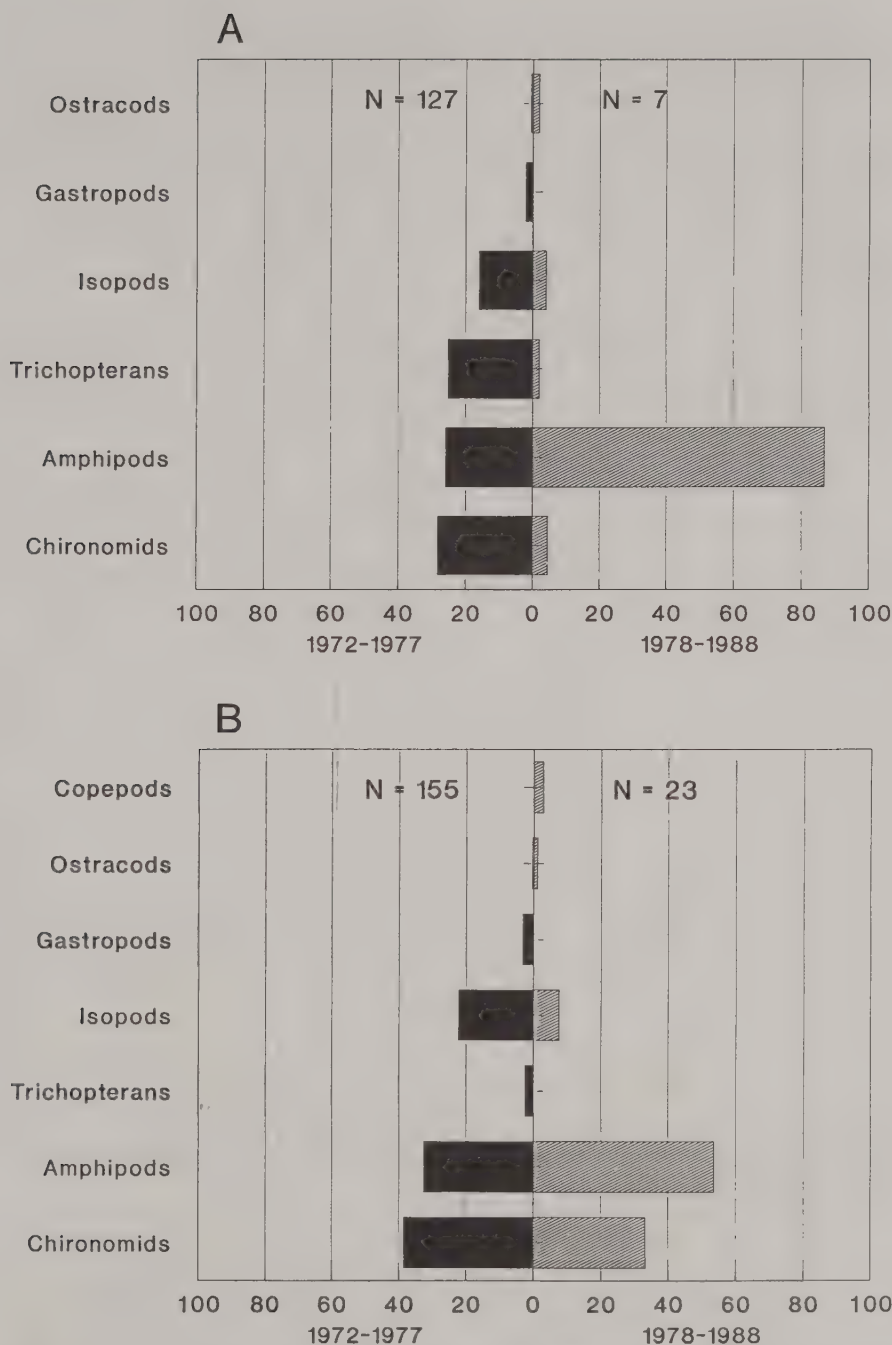


FIG. 4. Percent composition of white perch diet biomass by major taxa in 1972-77 (solid bars) and 1978-88 (striped bars) in the lower Bay of Quinte in (A) June-July and (B) August-September.

benthivores that was chironomids in any one year (by weight, with total sample size shown in parentheses) ranged as follows: yellow perch, 8.7-74.6% (717); trout-perch (*Percopsis omiscomaycus*), 29.3-64.0% (397); brown bullhead, 10.3-91.8% (505). These species, however, formed a major percentage of the biomass in the upper bay in only three years (1978, 1981, and 1984). The cumulative effect of these other benthivores may have been sufficient such that the hypothesis may have to be broadened to include them in the upper bay.

It is also possible that the habitat of the major chironomid species in the upper bay protects them to some degree from heavy predation. Most of the chironomid production there is confined to *C. plumosus* f. *semireductus*. This species is numerically very abundant in the first and second instars but is small in size (M. G. Johnson, pers. comm.). Although I have no direct measurements of the lengths of these instars, in two closely related species the lengths of the first instar ranged from 0.5 to 2 mm. The first and second instars of *C. plumosus* f.

TABLE 4. Calculated daily consumption (g AFDW/ha) of food items by white perch in the upper Bay of Quinte in the 1972-77 and 1978-88 periods for two summer intervals.

Food item	June-July			August-September		
	1972-77	1978-88	% change	1972-77	1978-88	% change
Chironomids	51.40	2.37	-95.4	43.49	3.40	-92.2
Cladocerans	1.07	1.27	+18.7	0.61	0.38	-37.7
Copepods	0.42	0.90	+114.3	4.05	3.90	-3.7
Amphipods	0.48	0.48	0	0.97	0.13	-86.6
Trichopterans	1.03	0.64	-37.9	0.34	0.02	-94.1
Gastropods	0.46	0.01	-97.8	1.14	0.01	-99.1

TABLE 5. Calculated daily consumption (g AFDW/ha) of food items by white perch in the lower Bay of Quinte in the 1972-77 and 1978-88 periods for two summer intervals.

Food item	June-July 1972-77	August-September		
		1972-77	1978-88	% change
Chironomids	0.91	7.37	0.12	-98.4
Copepods	0.04	0.02	0.08	+300.0
Amphipods	1.16	5.62	0.43	-92.3
Isopods	0.53	3.69	0.04	-98.9
Trichopterans	0.61	0.65	0	-100.0
Ostracods	0.08	0.24	0.03	-87.5
Gastropods	0.03	0.67	0	-100.0

TABLE 6. Daily net production (g AFDW/ha) of benthic macroinvertebrates in the Bay of Quinte and (in parentheses) the percentage of daily net production consumed by the white perch population.

	Upper bay				Lower bay			
	June-July		August-September		June-July		August-September	
	1972-77	1978-88	1972-77	1978-88	1972-77	1978-88	1972-77	1978-88
Chironomids	951.8 (5.4)	324.0 (0.7)	1187.9 (3.7)	404.4 (0.8)	2.4 (37.9)	—	4.4 (167.5)	63.1 (0.2)
Amphipods	—	—	—	—	9.1 (12.7)	—	5.0 (112.4)	139.0 (0.3)
Isopods	—	—	—	—	27.0 (2.0)	—	204.0 (1.8)	15.3 (0.3)

TABLE 7. Calculated daily net production (g AFDW/ha) of zooplankton forms in the May-September period in the upper and lower Bay of Quinte and (in parentheses) the percentage of daily net production consumed by the white perch population.

	Upper bay		Lower bay	
	1975-76	1979-88	1975-76	1979-88
Cladocerans	1685.5 (0.05)	1988.9 (0.04)	1430.5 (—)	2542.7 (—)
Calanoid copepods	—	11.1	—	16.8
Cyclopoid copepods	22.5 (9.93)	164.2 (1.37) ^a	42.3 (0.07)	156.5 (0.05) ^a

^aCalanoid and cyclopoid copepods combined.

semireductus are relatively short-lived, while the third and fourth instars, which can be up to 28 mm in length, live in U-shaped tubes and filter water through a net. These latter instars make a large contribution to the production but may not be heavily preyed upon because they are buried in the substrate. The first and second instars may be heavily utilized by benthivores, but these stages do not account for a high percentage of

the chironomid production (M. G. Johnson, pers. comm.). Chironomid population size, therefore, may not be regulated in any major way by white perch, which is consistent with the relatively small percentage of the chironomid production that they consume.

The scenario in the lower bay is much different than in the upper bay. The lower bay is much deeper (mean depth 23.8 m),

colder, and less eutrophic than the upper bay. The smaller mean length at age of white perch in the lower bay is a reflection of this difference. The lower bay exhibits significant currents generated by outflow from the upper bay and inflow from Lake Ontario (Freeman and Prinsenberg 1986). Nicholls and Hurley (1989) proposed that regular settling to the bottom of filamentous phytoplankton, *Melosira* in particular, during calm summer periods in the upper bay resulted in significant consumption of these filaments by benthic organisms. However, the continuous presence of strong currents in the lower bay would prevent this regular settling of filamentous algae. The link between white perch biomass and phytoplankton biomass in the lower bay was not tested, but the morphology and current regime of the lower bay would appear to make the relationship found in the upper bay unlikely.

Amphipods and isopods form a significant portion of the benthic community of the lower bay, along with chironomids (Johnson and McNeil 1986). Consumption of chironomids in 1972–77 relative to the net production was relatively high (38%) in June–July and exceeded production (168%) in August–September. Consumption of amphipods, mainly *P. hoyi*, exceeded the daily production in August–September 1972–77 (112%). Johnson and McNeil (1986) cited white perch predation as a factor in restricting *Pontoporeia* expansion in the lower bay. The data presented here support that hypothesis. In addition, *Pontoporeia* expanded in both numbers and area after 1977. The increased production of *Pontoporeia* in 1978–88 may be linked to the decline in white perch at that time when daily consumption by white perch was less than 1% of the daily net production.

Although the daily consumption data for the white perch population did not support the “benthic-link” hypothesis of Nicholls and Hurley (1989), it did support the “predation” hypothesis of Johnson and McNeil (1986) whereby white perch predation regulates the biomass of amphipods. Diet information and daily consumption calculations can therefore be used to test other hypotheses of the effects of predation on the production and standing stocks of prey organisms.

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References

- BLAXTER, J. H. S., B. B. PARRISH, AND W. DICKSON. 1964. The importance of vision in fish reaction to drift nets and trawls, p. 529. In *Modern fishing gear of the world*. Vol. 2. Second F.A.O. World Fishing Gear Congress, London, 1963.
- COOLEY, J. M., J. E. MOORE, AND W. T. GEILING. 1986. Population dynamics, biomass, and production of the macrozooplankton in the Bay of Quinte during changes in phosphorus loadings, p. 166–176. In C. K. Minns, D. A. Hurley, and K. H. Nicholls [ed.] *Project Quinte: point-source phosphorus control and ecosystem response in the Bay of Quinte, Lake Ontario*. Can. Spec. Publ. Fish. Aquat. Sci. 86: 270 p.
- CRAIG, J. F., A. SHARMA, AND K. SMILEY. 1986. The variability in catches from multi-mesh gillnets fished in three Canadian lakes. *J. Fish Biol.* 28: 671–678.
- ELLIOTT, J. M., AND L. PERSSON. 1978. The estimation of daily rates of food consumption for fish. *J. Anim. Ecol.* 47: 977–991.
- FREEMAN, N. G., AND S. J. PRINSEBERG. 1986. Exchange flows in the Adolphus Reach/North Channel, p. 27–39. In C. K. Minns, D. A. Hurley, and K. H. Nicholls [ed.] *Project Quinte: point-source phosphorus control and ecosystem response in the Bay of Quinte, Lake Ontario*. Can. Spec. Publ. Fish. Aquat. Sci. 86: 270 p.
- HALL, D. J., W. E. COOPER, AND E. E. WERNER. 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. *Limnol. Oceanogr.* 15: 839–928.
- HURLEY, D. A. 1986a. Fish populations of the Bay of Quinte, Lake Ontario, before and after phosphorus control, p. 201–214. In C. K. Minns, D. A. Hurley, and K. H. Nicholls [ed.] *Project Quinte: point-source phosphorus control and ecosystem response in the Bay of Quinte, Lake Ontario*. Can. Spec. Publ. Fish. Aquat. Sci. 86: 270 p.
- 1986b. Effect of nutrient reduction on the diets of four fish species in the Bay of Quinte, Lake Ontario, p. 237–246. In C. K. Minns, D. A. Hurley, and K. H. Nicholls [ed.] *Project Quinte: point-source phosphorus control and ecosystem response in the Bay of Quinte, Lake Ontario*. Can. Spec. Publ. Fish. Aquat. Sci. 86: 270 p.
- 1986c. Growth, diet, and food consumption of walleye (*Stizostedion vitreum vitreum*): an application of bioenergetics modeling to the Bay of Quinte, Lake Ontario, population, p. 224–236. In C. K. Minns, D. A. Hurley, and K. H. Nicholls [ed.] *Project Quinte: point-source phosphorus control and ecosystem response in the Bay of Quinte, Lake Ontario*. Can. Spec. Publ. Fish. Aquat. Sci. 86: 270 p.
- HURLEY, D. A., AND W. J. CHRISTIE. 1977. Depreciation of the warmwater fish community in the Bay of Quinte, Lake Ontario. *J. Fish. Res. Board Can.* 34: 1849–1860.
- JOHANSSON, O. E., AND J. BEAVER. 1983. Role of algae in the diet of *Chironomus plumosus* f. *semireductus* from the Bay of Quinte, Lake Ontario. *Hydrobiologia* 107: 237–247.
- JOHNSON, M. G. 1970. Production, energy flow and structure in some benthic macroinvertebrate associations of Lake Ontario. Ph.D. thesis, University of Toronto, Toronto, Ont. 182 p.
- JOHNSON, M. G., AND R. O. BRINKHURST. 1971. Production of benthic invertebrates of the Bay of Quinte and Lake Ontario. *J. Fish. Res. Board Can.* 28: 1699–1714.
- JOHNSON, M. G., AND O. E. MCNEIL. 1986. Changes in abundance and species composition in benthic macroinvertebrate communities of the Bay of Quinte, 1966–84, p. 177–189. In C. K. Minns, D. A. Hurley, and K. H. Nicholls [ed.] *Project Quinte: point-source phosphorus control and ecosystem response in the Bay of Quinte, Lake Ontario*. Can. Spec. Publ. Fish. Aquat. Sci. 86: 270 p.
- JOHNSON, T. B., AND D. O. EVANS. 1990. Size-dependent winter mortality of young-of-the-year white perch: climate warming and invasion of the Laurentian Great Lakes. *Trans. Am. Fish. Soc.* 119: 301–313.
- MCQUEEN, D. J., J. R. POST, AND E. L. MILLS. 1986. Trophic relationships in freshwater pelagic ecosystems. *Can. J. Fish. Aquat. Sci.* 43: 1571–1581.
- MINNS, C. K., AND D. A. HURLEY. 1986. Population dynamics and production of white perch, *Morone americana*, in the Bay of Quinte, Lake Ontario, p. 215–223. In C. K. Minns, D. A. Hurley, and K. H. Nicholls [ed.] *Project Quinte: point-source phosphorus control and ecosystem response in the Bay of Quinte, Lake Ontario*. Can. Spec. Publ. Fish. Aquat. Sci. 86: 270 p.
- NICHOLLS, K. H., L. HEINTSCH, E. CARNEY, J. BEAVER, AND D. MIDDLETON. 1986. Some effects of phosphorus loading reductions on phytoplankton in the Bay of Quinte, Lake Ontario, p. 145–158. In C. K. Minns, D. A. Hurley, and K. H. Nicholls [ed.] *Project Quinte: point-source phosphorus control and ecosystem response in the Bay of Quinte, Lake Ontario*. Can. Spec. Publ. Fish. Aquat. Sci. 86: 270 p.
- NICHOLLS, K. H., AND D. A. HURLEY. 1989. Recent changes in the phytoplankton of the Bay of Quinte, Lake Ontario: the relative importance of fish, nutrients, and other factors. *Can. J. Fish. Aquat. Sci.* 46: 770–779.
- RIDGWAY, M. S., D. A. HURLEY, AND K. A. SCOTT. 1990. Effects of winter temperature and predation on the abundance of alewife (*Alosa pseudoharengus*) in the Bay of Quinte, Lake Ontario. *J. Great Lakes Res.* 16: 11–20.
- SCHINDLER, D. W. 1969. Two useful devices for vertical plankton and water sampling. *J. Fish. Res. Board Can.* 26: 1948–1955.
- SHERI, A. N. 1968. Growth dynamics of white perch, *Roccus americanus*, during colonization of the Bay of Quinte, Lake Ontario. Ph.D. thesis, University of Waterloo, Waterloo, Ont. 366 p.
- STRUS, R. H., AND D. A. HURLEY. 1992. Interactions between alewife (*Alosa pseudoharengus*), their food, and phytoplankton biomass in the Bay of Quinte, Lake Ontario. *Int. Assoc. Great Lakes Res. J.* (In press)
- WIGGINS, G. B. 1977. Larvae of the North American caddisfly genera (Trichoptera). University of Toronto Press, Toronto, Buffalo, and London.
- WINDELL, J. T. 1978. Digestion and daily ration of fishes, chap. 7. In S. D. Gerking [ed.] *Ecology of freshwater fish production*. Blackwell Scientific Publications, Oxford.

Acute Handling Stress Alters Hepatic Glycogen Metabolism in Food-Deprived Rainbow Trout (*Oncorhynchus mykiss*)

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Acute handling stress resulted in significant elevation of plasma cortisol and lactate concentrations within 30 min in both fed and food-deprived rainbow trout (*Oncorhynchus mykiss*), indicating a typical stress response. Plasma glucose levels rose immediately (30 min) poststress in the food-deprived group, while there was a delayed response (2 h) in the fed group. The low liver glycogen content and total glycogen phosphorylase (GPase) and glycogen synthase (GSase) activities in the food-deprived group indicated an overall depression in glycogen metabolism. Acute handling stress maintained liver glycogen stores for up to 4 h in the food-deprived group, but the combined effects of limited substrate and increased energy demand necessitated mobilization of liver glycogen in the food-deprived group, but not in the fed group. This increased glycogen mobilization in the food-deprived group coincided with a secondary elevation in plasma cortisol concentration 4 h poststress. The results indicated that food-deprived rainbow trout were more sensitive to stress of handling and mobilized glycogen stores to meet the energy demand imposed by the stressor. The elevated plasma cortisol levels noted during acute handling stress could play an important role in energy partitioning, metabolically adapting the fish to handling stress.

Le stress aigu causé par la manipulation se traduit par une augmentation significative des concentrations plasmatiques de cortisol et de lactate dans les 30 min qui suivent chez la truite arc-en-ciel (*Oncorhynchus mykiss*) tant alimentée que privée de nourriture, ce qui est une réaction caractéristique du stress. Les concentrations plasmatiques de glucose ont augmenté immédiatement (30 min) après le stress dans le groupe privé de nourriture, tandis qu'on a observé un délai (2 h) dans la réponse du groupe de poissons alimentés. La faible teneur en glycogène hépatique et l'activité totale de la glycogène phosphorylase (GPase) et de la glycogène synthétase (GSase) dans le groupe privé de nourriture indiquaient un déclin global du métabolisme du glycogène. Le stress aigu causé par la manipulation a maintenu les réserves de glycogène hépatique pendant un maximum de 4 h dans le groupe privé de nourriture, mais les effets combinés du substrat limité et de la demande énergétique accrue ont nécessité la mobilisation du glycogène hépatique dans le groupe privé de nourriture, mais pas dans le groupe alimenté. Cette mobilisation accrue du glycogène dans le groupe privé de nourriture a coïncidé avec une augmentation secondaire de la concentration plasmatique de cortisol, 4 h après le stress. Les résultats indiquent que la truite arc-en-ciel privée de nourriture a été plus sensible au stress de la manipulation et a mobilisé ses réserves de glycogène pour répondre à la demande énergétique imposée par la source de stress. Les valeurs élevées de la concentration plasmatique de cortisol observées durant un stress aigu causé par la manipulation pourraient jouer un rôle important dans le fractionnement de l'énergie en permettant au poisson de s'adapter, sur le plan métabolique, au stress de manipulation.

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Handling is a common stress in fish aquaculture operations. Such a stressor elicits a primary stress response, which includes elevations in plasma cortisol and catecholamine levels (for reviews, see Mazeaud and Mazeaud 1981; Leatherland and Sonstegard 1984; Barton and Iwama 1991). These hormonal responses initiate a series of secondary stress responses, including alteration in glycogen and/or glucose metabolism, which enable the fish to avoid or cope with the maladaptive effects of the stressor (Mazeaud and Mazeaud 1981; Vijayan et al. 1991).

An important metabolic function of cortisol in teleosts appears to be the regulation of gluconeogenesis and/or glyconeogenesis (Vijayan et al. 1991). Elevated cortisol levels favour energy mobilization (Sheridan 1986; Vijayan et al. 1991), providing the fish with energy to respond to the immediate threat (Schreck 1981). The increased energy demand during stress must be met at the expense of anabolic processes, including growth (Pickering 1990; Vijayan et al. 1990).

Several studies have shown previously that stress affects the growth and performance of fish (for references, see Schreck 1981; Vijayan et al. 1990; Barton and Iwama 1991). Yet, little is known about the metabolic adaptation(s) of fish to stress. When brook trout (*Salvelinus fontinalis*) were stocked at high densities (chronic stressor), they exhibited lower growth rates and energy stores, indicating an increased energy demand (Vijayan et al. 1990). The ability to compensate for an additional stressor (such as acute handling) during chronic stress is impaired if the chronic stress had decreased food intake, thus leading to lower energy reserves (Vijayan et al. 1990).

Liver glycogen stores represent an important energy reserve, but to date, little is known about the temporal changes in glycogen metabolism during stress in fish. Previous studies have shown that hepatic glycogen levels decreased in stressed fish (Rush and Umminger 1978; Paxton et al. 1984; Vijayan et al. 1990). Since chronic cortisol treatment also decreased liver glycogen levels in teleosts (Davis et al. 1985; Barton et al. 1987;

Andersen et al. 1991; Vijayan et al. 1991), cortisol may exert an effect on stress-induced glycogen mobilization.

Nutritional state is known to modify glycogen metabolism in teleosts (see Sheridan and Mommensen 1991). Since the nutritional state also has a profound influence on both the stress response (Barton et al. 1988) and cortisol-induced changes in metabolism (Leach and Taylor 1982) of fish, any alteration in feeding (e.g. due to chronic stress) could modify the stress response and the resulting metabolic adaptation of the fish to an additional stressor.

The purpose of the present study, therefore, was to test two hypotheses: (1) that acute handling stress alters glycogen metabolism in rainbow trout and (2) that food deprivation modifies the primary stress response and glycogen metabolism to an acute handling stress in rainbow trout. Liver glycogen content and the activities of the hepatic enzymes, glycogen phosphorylase (GPase) and glycogen synthase (GSase), were used as indicators of changes in glycogen metabolism.

Materials and Methods

Experimental Animals

Rainbow trout (*Oncorhynchus mykiss*), obtained from Linwood Acres Trout Farm (Campbellcroft, Ontario), were maintained in indoor tanks (300 L) supplied with running, dechlorinated, and well-aerated city water at $7 \pm 1^\circ\text{C}$. The fish were maintained under a 12 h:12 h photoperiod and fed to satiation twice daily with commercial trout pellets (Purina Trout Chow). After 2 wk of acclimation (February 1991), all fish in this stock tank were weighed (in groups of five) and placed in experimental tanks.

Experimental Design

Groups of 20 fish (21 in one tank) (initial body weight 58.9 ± 3 g) were randomly assigned to four experimental tanks (200 L capacity) (two treatments each with a replicate) maintained under identical conditions to the stock tank. Two tanks were randomly chosen for the fed group and food was provided as before (feeding stopped 24 h prior to sampling), and the fish in the other two tanks were deprived of food during the 30-d experimental period.

Six fish were selected at random from each treatment for sampling (three fish from each tank) at the end of the 30-d period. The fish were quickly dip-netted and anaesthetized in a 1:10 000 (w/v) solution of neutralized aminobenzoic acid ethyl ester (MS222, Sigma Chemical Co.). Fish were bled rapidly by caudal puncture into heparinized syringes and the plasma, which was separated by centrifugation, was stored frozen at -80°C . The length and mass of each fish were established to determine condition factor. The liver was removed quickly, weighed (to determine hepatosomatic index, HSI), and freeze-clamped with Wollenberger tongs cooled in liquid nitrogen. Livers remained frozen for a few weeks at -80°C until analysed for glycogen content and GPase and GSase activities.

The remaining fish in the tanks were then subjected to a 3-min handling stress, which included chasing and scooping them in a net. The fish were sampled as above at 0.5 and 24 h poststress. At the other sampling times (1, 2, 4 and 8 h), five or six fish were removed from only one tank (no replicate), thereby alternating sampling among the duplicate tanks. This gave at least a few hours before the next sampling in each tank and prevented consecutive sampling from one tank.

The growth rate ([mean increase in body weight per day/body weight] $\times 100$), condition factor ([body weight in g]/[length

TABLE 1. Effect of food-deprivation on growth rate, condition factor, and HSI in rainbow trout; *significantly different from the fed group ($p < 0.05$, ANOVA).

	Fed	Food-deprived
Growth rate (% wt./day) ^a	0.6 ± 0.1 (2)	-0.38 ± 0.04 (2)*
Condition factor ^b	1.13 ± 0.01 (41)	0.94 ± 0.01 (40)*
HSI ^b	1.33 ± 0.03 (41)	0.87 ± 0.02 (40)*

^aValues represent mean $\pm$ SEM (n = total weight of all fish from two tanks).

^bValues represent mean $\pm$ SEM (n = 40 or 41; values were pooled because there were no significant differences between the time periods).

in cm^3) $\times 100$), and HSI [(liver weight in g)/(body weight in g)] $\times 100$) were calculated. After 30 d, the food-deprived group showed negative growth rate and significantly lower condition factor and HSI than the fed group, evidence that this group was fasted (Table 1).

Plasma cortisol concentrations were measured using a radioimmunoassay kit (ICN Biomedicals, Carson, CA) according to Andersen et al. (1991), while plasma glucose and liver glycogen contents were analysed enzymatically according to Foster and Moon (1989). Plasma lactate was enzymatically determined according to Bergmeyer (1983).

Hepatic enzyme activities were estimated according to Moon et al. (1989). Briefly, frozen liver pieces were sonicated (Kontes cell disrupter) in four volumes of a phosphorylation-dephosphorylation "stopping buffer" (Foster and Moon 1989) and centrifuged for 10 min at $12\,000 \times g$. The supernatant was applied to a Sephadex G-25 column (Moon et al. 1989), and the eluant was used for measuring enzyme activity at 10°C using a Beckman DU-65 recording spectrophotometer.

GPase Assay

A two-buffer system was used to distinguish GPase *a* from GPase total (*a* + *b*). The ratio of GPase activities with and without caffeine, but in the presence of AMP, represents the percentage of total GPase (*a* + *b*) in the active form (*a*) (% GPase *a*) (Moon et al. 1989). Enzyme activity is expressed in units (micromoles glucosyl units per minute) per gram tissue.

GSase Assay

Glycogen synthase activity was measured according to the method of Thomas et al. (1968). Briefly, the activity was measured at 15°C in the presence of 5 mM UDP-[U- ^{14}C]glucose (UDPG), 1% (w/v) glycogen, 1 mM EDTA, 20 mM glycylglycine, pH 7.4, and 10 mM Na_2SO_4 without (GSase *a*) or with 9 mM glucose 6-phosphate (GSase *a* + *b*). Enzyme activity is expressed in milliunits (nanomoles UDPG transformed to glycogen per minute under the assay conditions) per gram tissue. Results are presented as the percentage of total GSase (*a* + *b*) in the active form (*a*) (% GSase *a*).

Chemicals

Unless otherwise indicated, all biochemicals were obtained from Sigma Chemical Co., St. Louis, MO, or Boehringer Mannheim Co., Montreal, Que. All other reagents were purchased from local suppliers and were of the highest available purity.

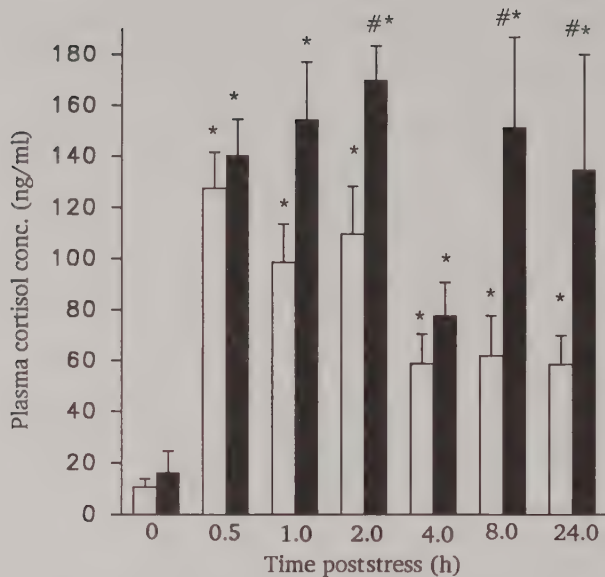


FIG. 1. Temporal changes in plasma cortisol concentrations after an acute handling stress in fed (open bar) and food-deprived (solid bar) rainbow trout. Values represent mean $\pm$ SEM ($n = 4-6$); *significantly different from the 0-h sampling period ($p < 0.05$, ANOVA); #significantly different from the fed group ($p < 0.05$, ANOVA).

Statistical Analysis

Data obtained were analysed statistically using two-way analysis of variance with treatment and time as factors. Where F values indicated significance ($p < 0.05$), means were compared using Tukey's test. There were no tank effects on any of the parameters tested at replicate sampling periods (0, 0.5, and 24 h poststress). Log-transformed data were used wherever necessary to satisfy homogeneity of variance, although non-transformed data are shown in the text.

Results

Plasma Parameters

There were no significant differences between the fed and food-deprived groups in any of the plasma parameters measured before acute handling stress (0 h sample; Fig. 1-3). Acute handling stress resulted in significant elevation of plasma cortisol levels within 30 min in both the fed and food-deprived groups, but remained significantly elevated above prestress levels (Fig. 1). After 4 h, plasma cortisol levels remained stable in the fed group for the rest of the sampling times, while the levels rose again in the food-deprived group and were significantly higher than in the fed group at 8 and 24 h poststress (Fig. 1). Two-way analysis of variance showed a significant treatment effect, with the food-deprived group having significantly higher cortisol levels than the fed group.

Plasma glucose levels increased significantly within 30 min poststress in the food-deprived group and remained elevated for 8 h, with the exception of a drop at 1 h poststress (Fig. 2). In the fed group, the rise in plasma glucose levels was more gradual and it was significantly above the prestress level only at 2 and 8 h poststress. Plasma glucose levels in the food-deprived group were significantly higher than in the fed group

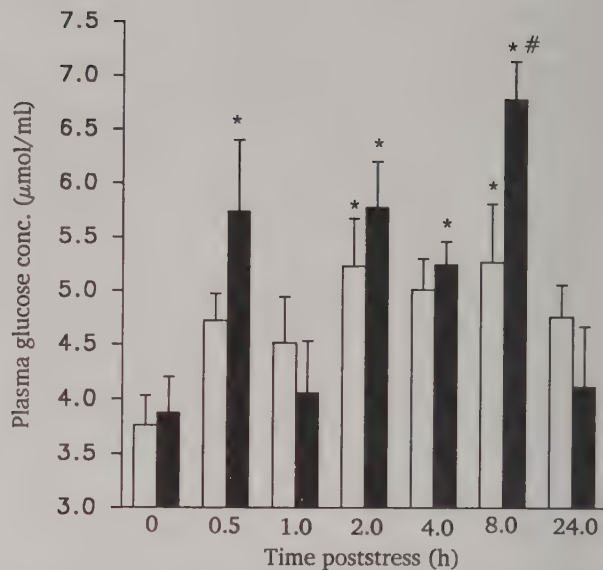


FIG. 2. Temporal changes in plasma glucose concentrations after an acute handling stress in fed (open bar) and food-deprived (solid bar) rainbow trout. Values represent mean $\pm$ SEM ($n = 4-6$); symbols as in Fig. 1.

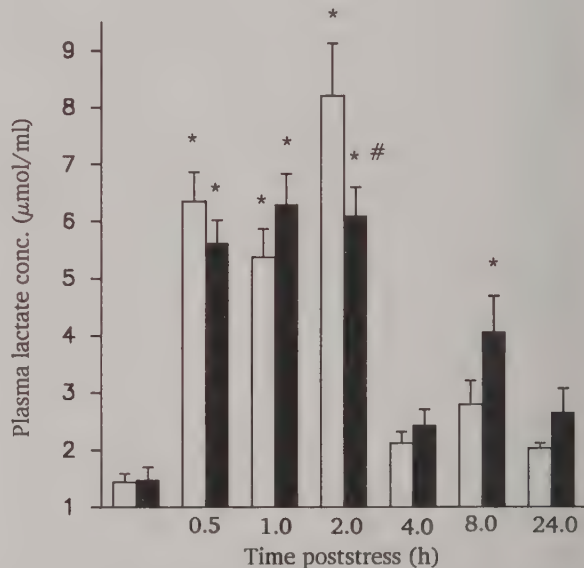


FIG. 3. Temporal changes in plasma lactate concentrations after an acute handling stress in fed (open bar) and food-deprived (solid bar) rainbow trout. Values represent mean $\pm$ SEM ($n = 5-6$); symbols as in Fig. 1.

at 8 h poststress after which the levels in both groups fell to levels which did not differ from prestress values.

Acute handling stress resulted in significant elevation of plasma lactate in both groups between 0.5 and 2 h poststress (Fig. 3). Plasma lactate levels dropped thereafter in both groups and were not significantly different from the prestress level in the fed group for the rest of the recovery period, but increased significantly in the food-deprived group at 8 h poststress. The

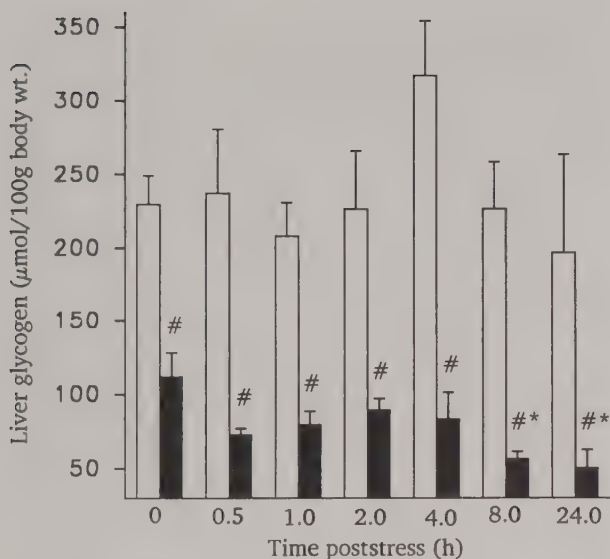


FIG. 4. Temporal changes in liver glycogen content as a function of body mass after an acute handling stress in fed (open bar) and food-deprived (solid bar) rainbow trout. Values represent mean $\pm$ SEM ($n = 4-6$); symbols as in Fig. 1.

food-deprived group had significantly lower plasma lactate than the fed group at 2 h poststress.

Liver Parameters

Food deprivation significantly lowered liver glycogen content in rainbow trout and this persisted at all time periods after the acute stress (Fig. 4). Handling stress did not alter liver glycogen content in the fed group over the 24 h period, while liver glycogen content in the food-deprived group dropped significantly at 8 and 24 h poststress.

The total hepatic activities of GPase and GSase were significantly lower in the food-deprived group than in the fed group (Table 2). There was a significant drop in total GPase activity at 0.5 h poststress in the fed group and at 8 h in both fed and food-deprived groups. Acute handling stress resulted in significant lowering of total GSase activity at 0.5 h in both treatment groups.

On the one hand, there was no significant difference in % GPase *a* (0-h sample; Fig. 5) between the fed and food-deprived groups prestress. On the other hand, % GSase *a* (0-h sample;

Fig. 6) was significantly higher in the food-deprived group, suggesting an increased glyconeogenic potential.

Acute handling stress resulted in a significant decrease in hepatic % GPase *a* in the fed group at 4 and 24 h poststress (Fig. 5). In the food-deprived group, % GPase *a* was significantly higher than the prestress value only at 8 h poststress and remained consistent throughout the other sampling times. When compared with the fed group, the food-deprived group had significantly lower % GPase *a* at 1 h and significantly higher % GPase *a* at 4, 8, and 24 h poststress, although these differences are due primarily to a change in the fed rather than the food-deprived group.

Percent GSase *a* showed a gradual increase post-stress in the fed group achieving significantly higher levels at 8 and 24 h poststress (Fig. 6). In the food-deprived group, % GSase *a* activity decreased significantly compared with the prestress level at 0.5 and 1 h poststress, after which the value showed an increasing trend reaching a significantly higher value at 4 h poststress and falling back to prestressed levels thereafter. At 4 h poststress, the food-deprived group had significantly higher % GSase *a* than the fed group (Fig. 6).

Discussion

The results from the present study, based on plasma and liver metabolite levels, clearly indicate that food deprivation increased the sensitivity of these trout to the stress of handling compared with the fed controls. Plasma cortisol and glucose concentrations are commonly used as indicators of primary and secondary stress responses to an acute stress in fish (see the introduction for references). The immediate elevation in plasma cortisol concentration following an acute stress seen in the present study supports the above statement. The secondary elevation (after 4 h) in plasma parameters occurred only in the food-deprived group and could potentially be due to other factors, including circadian rhythms. Several studies have demonstrated that salmonids exhibit circadian rhythms in plasma cortisol concentrations (Rance et al. 1982; Pickering and Pottinger 1983; Nichols and Weisbart 1984). Since the secondary elevation in plasma cortisol concentration did not occur in the fed fish, the absence of feeding may alter these endogenous rhythms in trout.

In the present study, there were no differences in any plasma parameters studied at 30 d of food deprivation (0-h sample; Fig. 1-3). Plasma cortisol and lactate levels did not change with 30 d of food deprivation in brook trout (Vijayan et al. 1991). Plasma glucose levels, however, are variable with food deprivation, showing either no change (Sheridan and Mommsen

TABLE 2. Temporal changes in hepatic total GPase and GSase activities after an acute handling stress in fed and food-deprived (FD) rainbow trout. Values represent mean $\pm$ SEM ($n = 4$ or 6); enzyme activity is expressed as micromoles glucosyl units per minute per 100 g body weight (taking HSI into account); *significantly different from the 0-h sampling period ($p < 0.05$, ANOVA).

Time (h)	GPase		GSase	
	Fed	FD ^a	Fed	FD ^a
0	3.65 $\pm$ 0.35	2.34 $\pm$ 0.04	0.41 $\pm$ 0.07	0.38 $\pm$ 0.05
0.5	2.80 $\pm$ 0.37*	2.70 $\pm$ 0.24	0.22 $\pm$ 0.02*	0.18 $\pm$ 0.02*
1.0	3.13 $\pm$ 0.26	2.65 $\pm$ 0.29	0.37 $\pm$ 0.09	0.27 $\pm$ 0.02
2.0	3.05 $\pm$ 0.11	2.46 $\pm$ 0.16	0.46 $\pm$ 0.06	0.41 $\pm$ 0.08
4.0	3.58 $\pm$ 0.30	2.16 $\pm$ 0.15	0.28 $\pm$ 0.03	0.45 $\pm$ 0.06
8.0	1.96 $\pm$ 0.21*	1.82 $\pm$ 0.16*	0.52 $\pm$ 0.13	0.26 $\pm$ 0.02
24.0	3.13 $\pm$ 0.17	2.19 $\pm$ 0.12	0.54 $\pm$ 0.06	0.34 $\pm$ 0.11

^aSignificantly different from the fed group ($p < 0.05$) at all sampling times.

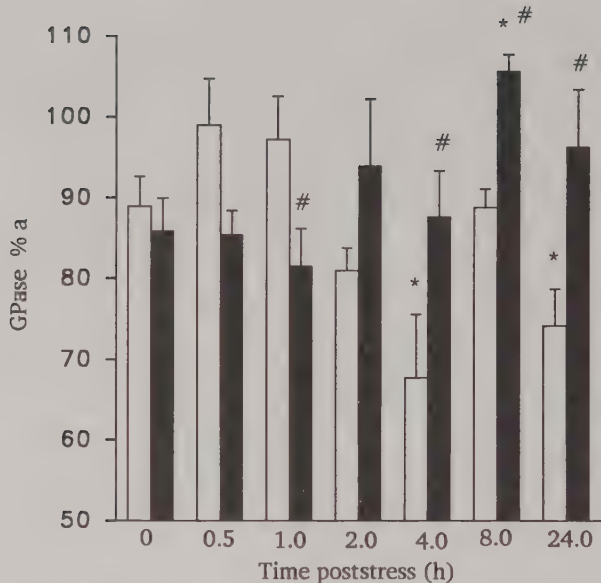


FIG. 5. Temporal changes in percent GPase *a* levels in liver after an acute handling stress in fed (open bar) and food-deprived (solid bar) rainbow trout. Values represent mean $\pm$ SEM ($n = 5-6$); symbols as in Fig. 1.

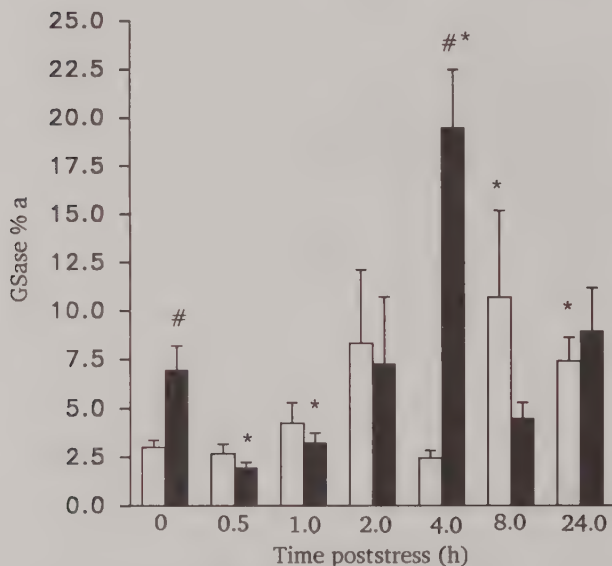


FIG. 6. Temporal changes in percent GSase *a* levels in liver after an acute handling stress in fed (open bar) and food-deprived (solid bar) rainbow trout. Values represent mean $\pm$ SEM ($n = 5-6$); symbols as in Fig. 1.

1991; present study) or a decrease (Barton et al. 1988; Moon et al. 1989; Foster and Moon 1991; Vijayan et al. 1991) with food deprivation. Several factors might be responsible for this variable glucose response, including differences in species, age, season, temperature, length of fast, and prior rearing of the fish (Suarez and Mommsen 1987).

The higher plasma cortisol levels in the food-deprived group could be attributed to several possibilities. First, the interrenal tissue may be sensitized to the stressor in the food-deprived

group, resulting in higher cortisol secretion rates. It has been shown previously in both brook trout and coho salmon (*Oncorhynchus kisutch*) that interrenal tissue from fish subjected to a stressor (high stocking density) had higher cortisol secretion rates (Patino et al. 1986; Vijayan and Leatherland 1990). Second, the clearance of cortisol may be attenuated by food deprivation, resulting in consistently higher levels of cortisol concentration (Fig. 1). This, however, seems unlikely, as the levels decreased at 4 h in both groups, suggesting a similar clearance mechanism. The explanation for the fall in plasma cortisol concentration at 4 h is not clear, but could be due to increased liver uptake and catabolism (Vijayan and Leatherland 1990). It seems, therefore, that with food deprivation the secretion of cortisol may far exceed the clearance, resulting in elevated plasma cortisol levels. Plasma cortisol levels remained elevated in both groups even at 24 h poststress (compared with the prestress levels). This result is consistent with that of Barton et al. (1987), where plasma cortisol levels remained elevated up to 24 h after a 30-s handling stress in juvenile rainbow trout. Another possible explanation for the elevated cortisol levels may be the density changes associated with fish removal after each sampling. Although the handling stress elevated plasma cortisol levels in both groups, the effects of stress on hepatic glycogen metabolism were evident only in the food-deprived group, suggesting that nutritional state of the animal modifies the stress response (Barton et al. 1988).

Plasma lactate increased with stress in both groups (Fig. 3), peaking at about 2 h, which is in agreement with studies on exercise in teleosts (Wood and Perry 1985). The transient increase in plasma lactate at 8 h in the food-deprived group indicates an increased stress response, in concert with both plasma cortisol (Fig. 1) and glucose (Fig. 2) increases. However, it is yet to be ascertained if cortisol has any direct role in this secondary lactate release. Cortisol may also modulate the rate of utilization of lactate. Earlier studies have indicated both an increased oxidation in fish treated with cortisol (Chan and Woo 1978) and an increase in the rate of gluconeogenesis from lactate in hepatocytes incubated with cortisol (Renaud and Moon 1980).

The increased plasma glucose level in rainbow trout after an acute handling stress could potentially be due to the action of cortisol. It has been shown that plasma cortisol and glucose levels increase with acute stress in fish (for references, see Barton and Iwama 1991). Moreover, several studies have shown that cortisol increased the gluconeogenic capacity in teleosts (for references, see Vijayan et al. 1991), although plasma glucose levels are variable and are not solely indicative of an increased cortisol concentration. The reason for this discrepancy between plasma cortisol and glucose may be related to an altered glucose turnover, and if so, plasma glucose levels are not a good indicator of long-term glucose metabolism (Suarez and Mommsen 1987).

Glucose elevation in the food-deprived group was immediate after handling stress (within 30 min) as opposed to a delayed response (2 h) in the fed group (Fig. 2). This rapid elevation in plasma glucose in the food-deprived group could be due to the action of catecholamines. Under acute stress, catecholamines are released rapidly, directing the phosphorylation of the inactive form of GPase, resulting in increased glycogenolysis in fish (Perry and Wood 1989; Wright et al. 1989). Surprisingly, there was no effect on % GPase *a* in the food-deprived group at 30 min. One possible explanation may be that % GPase *a* was already under maximum or near maximum stimulation (>80%) (Fig. 5) and was adequate to provide the necessary

plasma glucose. The changes in plasma glucose in the absence of changes in % GPase *a* at 30 min suggest that some other mechanism(s) may be regulating glucose availability in this species during acute stress. It may also be that the phosphorylation and dephosphorylation of GSase plays an important regulating role in the shift in metabolic flux. The significant lowering of % GSase *a* at 30 min in the food-deprived group implies a reduction in the glycconeogenic capacity poststress. This response, however, was not seen in the fed group, implying that food deprivation may modify the response of liver to catecholamine action. Reid et al. (1992) have recently demonstrated that chronic cortisol treatment alters β -receptor dynamics in rainbow trout hepatocytes, making them more sensitive to catecholamine action. From the present result, it appears that the nutritional state may either directly influence catecholamine responsiveness or indirectly favour catecholamine sensitivity through the elevation of cortisol concentration. Further work is necessary before the process can be clearly explained.

This study is the first to examine the temporal changes in glycogen metabolism in response to an acute handling stress in a teleost. The results clearly indicate that handling stress mobilizes hepatic glycogen stores to a greater extent in food-deprived rainbow trout than in fed fish.

The increased % GSase *a* in the food-deprived group prior to stress (0-h sample) suggests that glycconeogenesis is enhanced to replenish glycogen stores should adequate substrate be available. The negative growth rate and lower condition factor in the food-deprived group at 30 d (Table 1) support the idea that at least body protein mobilized in food-deprived rainbow trout is utilized to provide some glycogen synthesis in order to conserve the glycogen which does exist (Sheridan and Mommsen 1991). The low liver glycogen content together with decreased total activities of both GPase and GSase in the food-deprived group indicates a reduction in glycogen metabolism (Fig. 4; Table 2). Thus, their metabolic potential was severely compromised compared with the fed fish. Foster and Moon (1991) have shown an overall depression in the hepatic metabolic potential of yellow perch (*Perca flavescens*) during fasting. In such a situation the fish may not have been able to maintain glycogen stores at the expense of other substrates; any additional demand may have led to increased glycogen mobilization. Despite this, the food-deprived fish did maintain their glycogen stores initially at a level not significantly different from the prestress level, possibly by glycconeogenesis as the 4-h increase in % GSase *a* (Fig. 6) seems to indicate. Due to their reduced metabolic reserves (Table 1), however, the substrate available to fuel metabolic demands cannot be maintained by breakdown of proteins and lipids alone, resulting in enhanced liver glycogen mobilization at 8 and 24 h (Fig. 4). This increase in mobilization is associated with an increased % GPase *a* (Fig. 5). If another stress or a more substantial stress were to occur, one questions whether these fish could survive.

Acute handling stress had no effect on liver glycogen content in the fed group. Since the fed fish are in a positive energy balance, as shown by their growth rate and condition factor (Table 1), they have abundant energy reserves for mobilization. When exposed to a stressor, the fish probably rely primarily on lipids and proteins for their energy needs as well as for replenishing their glycogen stores. Elevated cortisol levels poststress might favour lipid and protein mobilization for energy and gluconeogenesis (Foster and Moon 1986; Vijayan et al. 1991), thereby allowing the fish to cope with the immediate stress without depleting glycogen stores (Sheridan and

Mommsen 1991). In fact, decreases in % GPase *a* at 4 and 24 h (Fig. 5) and increases in % GSase *a* at 8 and 24 h (Fig. 6) would assist in the restoration of any glycogen which may have been used during early periods of the stress.

It is possible that cortisol is playing a direct role in glycogen mobilization under acute stress situations. Recently, treatment of brook trout with RU486 (a glucocorticoid antagonist) resulted in significant elevation of liver glycogen content, suggesting that cortisol might have a direct effect on hepatic glycogen metabolism (Vijayan and Leatherland 1992). Even in the present study, increased glycogen mobilization in the food-deprived group poststress coincided with a secondary elevation in plasma cortisol levels 4 h poststress (Fig. 1 and 4). The change in glycogen mobilization was observed only in the food-deprived group, indicating that the nutritional state of the animal influences the cortisol response in this species (Leach and Taylor 1982; Vijayan et al. 1991).

In conclusion, the results indicate that food deprivation in rainbow trout is associated with a more severe metabolic effect to acute handling stress than the fed group. Liver glycogen content may be maintained just after handling stress by enhanced glycconeogenesis in the food-deprived group. However, the recovery process in the food-deprived group requires far more energy than in the fed group because of its low metabolic reserves, resulting in glycogen depletion. Liver glycogen content may be an important factor regulating the performance of the fish because of its utilization for immediate energy requirement. Long-term depletion of hepatic glycogen stores might be detrimental to the fish because metabolically they are less capable of tolerating an additional stressor. Cortisol appears to be an important glucoregulatory hormone in rainbow trout, mobilizing energy stores to compensate for the demand imposed by the handling stress during food deprivation.

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References

- ANDERSEN, D. E., S. D. REID, T. W. MOON, AND S. F. PERRY. 1991. Metabolic effects associated with chronically elevated cortisol in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 1811-1817.
- BARTON, B. A., AND G. K. IWAMA. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish Dis. 1: 3-26.
- BARTON, B. A., C. B. SCHRECK, AND L. D. BARTON. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. Dis. Aquat. Org. 2: 173-185.
- BARTON, B. A., C. B. SCHRECK, AND L. G. FOWLER. 1988. Fasting and diet content affect stress-induced changes in plasma glucose and cortisol in juvenile chinook salmon. Prog. Fish-Cult. 50: 16-22.
- BERGMEYER, H. U. [ED.] 1983. Methods of enzymatic analysis. Vol. VI. 3rd ed. Verlag Chemie, Weinheim. p. 163-172 and 582-588.
- CHAN, D. K. O., AND N. Y. S. WOO. 1978. Effect of cortisol on the metabolism of the eel, *Anguilla japonica*. Gen. Comp. Endocrinol. 35: 205-215.
- DAVIS, K. B., P. TORRANCE, N. C. PARKER, AND M. A. SUTTLE. 1985. Growth, body composition and hepatic tyrosine aminotransferase activity in cortisol-fed channel catfish, *Ictalurus punctatus* Rafinesque. J. Fish Biol. 27: 177-184.
- FOSTER, G. D., AND T. W. MOON. 1986. Cortisol and liver metabolism of immature American eels, *Anguilla rostrata* (LeSueur). Fish Physiol. Biochem. 1: 113-124.

1989. Insulin and the regulation of glycogen metabolism and gluconeogenesis in American eel hepatocytes. *Gen. Comp. Endocrinol.* 73: 374-381.
1991. Hypometabolism with fasting in the yellow perch (*Perca flavescens*): a study of enzymes, hepatocyte metabolism, and tissue size. *Physiol. Zool.* 64: 259-275.
- LEACH, G. J., AND M. H. TAYLOR. 1982. The effects of cortisol treatment on carbohydrate and protein metabolism in *Fundulus heteroclitus*. *Gen. Comp. Endocrinol.* 48: 76-83.
- LEATHERLAND, J. F., AND R. A. SONSTEGARD. 1984. Pathobiological responses of feral teleosts to environmental stressors: interlake studies of the physiology of Great Lake salmon, p. 116-149. In J. O. Nriagu, V. Cairns, and P. Hodson [ed.] *Monitoring contaminant effects on fisheries*. Wiley, Toronto, Ont.
- MAZEAUD, M. M., AND F. MAZEAUD. 1981. The role of catecholamines in the stress response of fish, p. 49-75. In A. D. Pickering [ed.] *Stress and fish*. Academic Press, London.
- MOON, T. W., G. D. FOSTER, AND E. M. PLISetskaya. 1989. Changes in peptide hormones and liver enzymes in the rainbow trout deprived of food for 6 weeks. *Can. J. Zool.* 67: 2189-2193.
- NICHOLS, D. J., AND M. WEISBART. 1984. Plasma cortisol concentrations in Atlantic salmon, *Salmo salar*: episodic variations, diurnal change, and short term response to adrenocorticotrophic hormone. *Gen. Comp. Endocrinol.* 56: 169-176.
- PATINO, R., C. B. SCHRECK, J. L. BLANKS, AND W. S. ZAUGG. 1986. Effects of rearing conditions on the developmental physiology of smolting coho salmon. *Trans. Am. Fish. Soc.* 115: 828-837.
- PAXTON, R., D. H. GIST, AND B. L. UMMINGER. 1984. Serum cortisol levels in thermally-acclimated goldfish (*Carassius auratus*) and killifish (*Fundulus heteroclitus*): implications in control of hepatic glycogen metabolism. *Comp. Biochem. Physiol. B* 8: 813-816.
- PERRY, S. F., AND C. M. WOOD. 1989. Control and coordination of gas transfer in fishes. *Can. J. Zool.* 67: 2961-2970.
- PICKERING, A. D. 1990. Stress and the suppression of somatic growth in teleost fish, p. 473-479. In A. Eppler, C. G. Scanes, and M. H. Stetson [ed.] *Progress in comparative endocrinology*. Wiley-Liss, Inc., New York, NY.
- PICKERING, A. D., AND T. G. POTTINGER. 1983. Seasonal and diel changes in plasma cortisol levels of the brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 49: 232-239.
- RANCE, T. A., B. I. BAKER, AND G. WEBLEY. 1982. Variations in plasma cortisol concentrations over a 24-hour period in the rainbow trout *Salmo gairdneri*. *Gen. Comp. Endocrinol.* 48: 269-274.
- REID, S. D., T. W. MOON, AND S. F. PERRY. 1992. Rainbow trout (*Oncorhynchus mykiss*) hepatocyte β -adrenoceptors, catecholamine responsiveness and the effects of cortisol. *Am. J. Physiol.* 262: R794-R799.
- RENAUD, J. M., AND T. W. MOON. 1980. Characterization of gluconeogenesis in hepatocytes isolated from the American eel, *Anguilla rostrata* Lesueur. *J. Comp. Physiol. B* 135: 115-125.
- RUSH, S. B., AND B. L. UMMINGER. 1978. Elimination of stress-induced changes in carbohydrate metabolism of goldfish (*Carassius auratus*) by training. *Comp. Biochem. Physiol. A* 60: 69-73.
- SCHRECK, C. B. 1981. Stress and compensation in teleostean fishes: response to social and physical factors, p. 295-321. In A. D. Pickering [ed.] *Stress and fish*. Academic Press, London.
- SHERIDAN, M. A. 1986. Effects of thyroxine, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, *Oncorhynchus kisutch*, during smoltification. *Gen. Comp. Endocrinol.* 64: 220-238.
- SHERIDAN, M. A., AND T. P. MOMMSEN. 1991. Effects of nutritional state on in vivo lipid and carbohydrate metabolism of coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 81: 473-483.
- SUAREZ, R. K., AND T. P. MOMMSEN. 1987. Gluconeogenesis in teleost fishes. *Can. J. Zool.* 65: 1869-1882.
- THOMAS, S. A., K. K. SCHLENDER, AND J. LARNER. 1968. A rapid filter paper assay for UDPG glucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-¹⁴C-glucose. *Anal. Biochem.* 25: 486-499.
- VIJAYAN, M. M., J. S. BALLANTYNE, AND J. F. LEATHERLAND. 1990. High stocking density alters the energy metabolism of brook charr, *Salvelinus fontinalis*. *Aquaculture* 88: 371-381.
1991. Cortisol-induced changes in some aspects of the intermediary metabolism of *Salvelinus fontinalis*. *Gen. Comp. Endocrinol.* 82: 476-486.
- VIJAYAN, M. M., AND J. F. LEATHERLAND. 1990. High stocking density affects cortisol secretion and tissue distribution in brook charr, *Salvelinus fontinalis*. *J. Endocrinol.* 124: 311-318.
1992. In vivo effects of the steroid analogue RU486 on some aspects of intermediary and thyroid metabolism of brook charr, *Salvelinus fontinalis*. *J. Exp. Zool.* 263: 265-271.
- WOOD, C. M., AND S. F. PERRY. 1985. Respiratory, circulatory, and metabolic adjustments to exercise in fish, p. 2-22. In R. Gilles [ed.] *Circulation, respiration, metabolism*. Springer-Verlag, Berlin.
- WRIGHT, P. A., S. F. PERRY, AND T. W. MOON. 1989. Regulation of hepatic gluconeogenesis and glycogenolysis by catecholamines in rainbow trout during environmental hypoxia. *J. Exp. Biol.* 147: 169-188.

Influence of Dietary Protein and Lipid on Nitrogen and Energy Losses in Lake Trout, *Salvelinus namaycush*

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Jayaram, M. G., and F. W. H. Beamish. 1992. Influence of dietary protein and lipid on nitrogen and energy losses in lake trout, *Salvelinus namaycush*. Can. J. Fish. Aquat. Sci. 49: 2267–2272.

Nitrogen and energy loss in fecal and nonfecal excretions by lake trout, *Salvelinus namaycush* (171–268 g), varied directly with dietary protein (40 and 50% dry weight) and inversely with lipid (10 and 20%) among four test diets. Diets high in lipid increased dry matter digestibility resulting in reduced nitrogen and energy loss in feces. $\text{NH}_3\text{-N}$ constituted 74.0–93.0% of total N excreted in fasted (4–12 d) and fed trout, while urea-N constituted 4.6–17.8% of total N. Excretion of urea-N was high in fish fed the high-lipid diet, particularly when dietary protein was also high. Total N and $\text{NH}_3\text{-N}$ excretion rose to peak values between 4 and 12 h after feeding and thereafter declined to prefeeding levels by 24 h. Urea-N excretion rates varied significantly following ingestion of all but one (50% protein, 20% lipid) test diet, reaching peak values between 4 and 12 h. In fasted fish, total N and $\text{NH}_3\text{-N}$ excretions were significantly low and failed to show a daily pattern; the urea-N excretion rate was significantly high compared with fish fed diets 1, 2, and 3 and followed a daily pattern of change. On average, a larger proportion (13–25%) of food energy is lost through nitrogenous excretion than in the feces (8–22%).

La perte d'azote et d'énergie dans les excréments fécaux et non fécaux du touladi, *Salvelinus namaycush* (171 à 268 g), varie en raison directe de la fraction de protéines dans l'alimentation (40 et 50 % en poids sec) et en raison inverse de la fraction de lipides (10 et 20 %) avec quatre régimes alimentaires expérimentaux. Les régimes alimentaires à teneur élevée en lipides augmentent la digestibilité des matières sèches, ce qui se traduit par une diminution de la perte d'azote et d'énergie dans les excréments. L'azote sous forme de NH_3 représente entre 74,0 et 93,0 % de l'azote total excrété chez les truites à jeun (pendant 4 à 12 jours) et chez les truites alimentées, tandis que l'azote sous forme d'urée représente entre 4,6 et 17,8 % de l'azote total. L'excrétion d'azote sous forme d'urée est élevée chez les poissons ayant reçu le régime alimentaire à teneur élevée en lipides, en particulier lorsque la teneur en protéines alimentaires est également élevée. L'excrétion d'azote total et d'azote sous forme de NH_3 augmente jusqu'à un maximum entre 4 et 12 h après l'alimentation des poissons et décline ensuite jusqu'aux niveaux pré-alimentation à 24 h. Les taux d'excrétion d'azote sous forme d'urée varient de manière significative après l'ingestion dans tous les régimes alimentaires expérimentaux sauf un (50 % protéines, 20 % lipides) et atteignent un maximum entre 4 et 12 h. Chez les poissons à jeun, les excréments d'azote total et d'azote sous forme de NH_3 sont très faibles et on n'a observé aucun profil de variation journalière; le taux d'excrétion d'azote sous forme d'urée est très élevé par rapport à celui des poissons ayant reçu les régimes 1, 2 et 3 et suit un profil de variation journalière. En moyenne, la proportion d'énergie alimentaire perdue est plus importante par l'excrétion azotée (13 à 25 %) que par les excréments (8 à 22 %).

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Dietary composition strongly influences the quantity and chemical form of nitrogen excreted by fish (Savitz 1971). The more protein that is utilized as an energy source, the more nitrogen that is excreted, generally as ammonia and urea (Goldstein and Forster 1970; Cho and Kaushik 1985). Dietary protein is an important contributor to the energy requirements of carnivorous fish (van den Thillard 1986). Salmonids cannot effectively use carbohydrate; therefore, dietary lipid is commonly used in fish feed formulation (Cowey 1980) to "spare" the deamination and oxidation of protein as an energy source.

Nitrogen is lost primarily through feces and metabolic excretion (Soofani and Hawkins 1985). Nitrogenous excretion may vary from 2 to 12% of the energy intake (Brett and Groves 1979). Ammonia, which is largely formed in the liver, requires no modification and hence no energy for excretion. In contrast, urea synthesis and excretion expends energy (Goldstein and Forster 1970). As a result the component of metabolizable energy available to fish is influenced by both the magnitude and composition of nitrogenous excretions (Cho and Kaushik

1985) which in turn are dependent on dietary composition (Dabrowski et al. 1987). Postprandial patterns of nitrogenous excretion not only correspond to the rate of evacuation of food but also indicate the pattern of dietary protein utilization (Polunin and Koike 1987). Because over 90% of the nitrogen eliminated from the body is of dietary origin (Leid and Braaten 1984), the fecal and nonfecal losses of nitrogen and energy together represent inefficiencies in dietary protein utilization.

Natural diets generally result in lower conversion efficiencies than formulated diets due to higher fecal and nonfecal wastage of nutrients (Elliott 1976). Several workers have demonstrated the protein "sparing" action of dietary lipid (Atherton and Aitken 1970; Reinitz et al. 1978), but only a few have systematically evaluated the effects of dietary protein-lipid interactions on nutrient digestibilities and nitrogenous losses in fish (Beamish and Thomas 1984; Watanabe et al. 1987). The main purpose of this study was to investigate the dietary protein and lipid effects on the composition of postprandial nitrogenous (ammonia + urea) excretion and fecal nitrogen losses in lake trout, *Salvelinus namaycush*.

Materials and Methods

Hatchery-reared lake trout were held in circular fiberglass tanks (2400 L) supplied with a continuous flow of aerated non-chlorinated groundwater ($10 \pm 1^\circ\text{C}$). Dissolved oxygen and ammonia ($\text{NH}_3 + \text{NH}_4^+$) were maintained above and below 8 and $0.1 \text{ mg}\cdot\text{L}^{-1}$, respectively. Daily photoperiod was 15 h (08:00–23:00), with dawn and dusk being simulated by a half hour of brightening or dimming. Fish were fed to satiation once daily (09:00) with a commercial formulated diet for trout (GRT-78G; Martin Feed Mills Ltd., Ontario) until the experiment commenced.

Experiments were conducted between April and December 1987 in metabolic chambers which permitted separate collection of fecal and nonfecal excretions (Beamish and Thomas 1984). A constant flow ($76\text{--}84 \text{ mL}\cdot\text{min}^{-1}$) of oxygen-enriched water to each chamber assured a dissolved oxygen concentration of over 90% of air saturation. Chambers were submerged in a temperature-controlled water bath ($13 \pm 1^\circ\text{C}$) to maintain the preferred temperature of $12 \pm 1^\circ\text{C}$ for lake trout (McCauley and Tait 1970).

Experiment 1

Individual fish ($n = 11$; $216 \pm 25 \text{ g}$; mean $\pm$ SD) were anesthetized ($60 \text{ mg MS222}\cdot\text{L}^{-1}$), weighed ($\pm 1.0 \text{ g}$), and placed in metabolic chambers 15 d before an experiment was begun. During this time, fish were fed to satiation once daily at 10:00 one of the four experimental diets. The test diets were composed of varying amounts of the same components such that the two levels of proteins (40 and 50%) were factorially combined with two levels of lipids (10 and 20%; Table 1). Following the 15-d acclimation period, fish were deprived of food for 2 d before commencement of an experiment. Each of the four test diets was fed to 11 fish in random order at 0.75% of live body weight per day for nine consecutive days. A daily

ration level of 0.75% live body weight was selected to avoid contamination of nitrogenous losses by excess feed. This was based on a preliminary feeding experiment in which ad libitum intake of trout pellets (GRT-78G) over 15 min by lake trout ($n = 8$; $214 \pm 23 \text{ g}$) housed individually in metabolic chambers equalled $0.78 \pm 17\%$ body weight. The daily postprandial pattern of nitrogenous excretion "stabilized" by the third day of feeding. Hence, chamber water samples and feces were not collected and analysed until the fourth day of restricted feeding.

Aliquots of chamber water (20 mL) were collected automatically at 20 min intervals for 24 h following presentation of the daily ration into a vessel containing 0.5 mL of concentrated sulphuric acid to prevent nitrogen loss (Sayer and Davenport 1987). Feces that accumulated in a trough fitted to the bottom of a chamber over the corresponding period were removed through a drainage tube. Before the presentation of the next day's ration (10:00), water and fecal samples collected over the preceding 24 h were removed for subsequent analyses. Measurements of total N, ammonia-N ($\text{NH}_3\text{-N}$) ($\text{NH}_3 + \text{NH}_4^+$), and urea-N in water samples from the chambers containing fish were adjusted on the basis of equivalent nitrogen concentration in water from a chamber without fish.

Total N was determined from a 100-mL aliquot of chamber water by the Kjeldhal technique (AOAC 1980). $\text{NH}_3\text{-N}$ was determined by the Solorozano (1969) colorimetric technique using phenolhypochlorite reagent. Urea-N was measured by the diacetyl monoxime method (Crocker 1967). Creatinine (Slot 1965) was measured early in the study but was present only in trace amounts and subsequent analyses were discontinued. Protein content ($\text{Kjeldhal N} \times 6.25$) of test diets and feces was measured after acid digestion (AOAC 1980). Following alkalization ($\text{pH} > 12$) with 10 M sodium hydroxide, measurement of total N and $\text{NH}_3\text{-N}$ by a probe (model 95-12, Orion Research Inc.) was carried out by spiking each sample with ammonium sulphate ($200 \mu\text{g}$ of N). Lipid was measured after extraction in

TABLE 1. Composition of experimental test diets formulated by J. Hilton, Department of Nutrition, University of Guelph, Guelph, Ont.

	Diet (% weight)			
	1	2	3	4
Ingredient				
Fish meal (Capelin, 68% CP) ^a	30	30	30	30
Soybean meal (40% CP)	20	20	20	20
Wheat middlings (15% CP, 8% fiber)	5	5	5	5
Fish oil (herring with antioxidant)	3.4	17.4	3.4	17.4
Gelatin	14.1	14.1	23.5	23.5
Cellulose	11.8	4.7	7.0	0.1
Kaolin	12.4	5.4	7.85	0.75
Vitamin premix ^b	2	2	2	2
Mineral premix ^b	1	1	1	1
Methionine	0.4	0.4	0.25	0.25
Composition				
Crude protein ^c	39.57	41.34	50.12	50.74
Lipid ^c	9.90	21.10	9.95	21.54
Ash ^c	16.6	10.42	12.08	6.34
Water	7.6	4.8	7.9	5.1
Gross energy ^d ($\text{J}\cdot\text{g}^{-1}$)	17874	22007	19089	22299
Digestible energy ^d ($\text{J}\cdot\text{g}^{-1}$)	13338	18256	15797	21007
Energy:protein ($\text{J}\cdot\text{g}^{-1}$)	44973	53225	38054	43972

^aCP, crude protein.

^bRefer to Hilton and Slinger (1981) for composition of vitamin and mineral supplements.

^cAverage of six values.

^dAverage of two values.

TABLE 2. Nitrogen loss and energy loss (in parentheses) in lake trout in relation to concentration of dietary protein and lipid. Each value is expressed on the basis of a 1-kg lake trout and represents the mean $\pm$ SD of 11 fish (216.0 ± 25.0 g) for each diet. Values in a column not sharing the same superscript were significantly different at $P < 0.05$.

Diet (protein/lipid) (% dry weight)	Nitrogen intake ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) (Energy intake ($\text{J} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$))	Nitrogen loss ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) (Energy loss ($\text{J} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$))			Fecal total N (Gross energy)
		Total N	Nonfecal $\text{NH}_3\text{-N}$	Urea-N	
40/10	478.4 ± 7.3^a (23657 $\pm$ 54 ^a)	366.1 ± 17.7^a	340.9 ± 7.4^a (8464 $\pm$ 134 ^a)	17.2 ± 2.9^a (397 $\pm$ 28 ^a)	28.2 ± 2.8^a (369 $\pm$ 16 ^a)
40/20	498.6 ± 11.6^a (29676 $\pm$ 115 ^b)	339.2 ± 16.4^b	308.1 ± 6.1^b (7702 $\pm$ 124 ^b)	19.7 ± 3.1^a (495 $\pm$ 160 ^a)	18.3 ± 1.1^b (4777 $\pm$ 33 ^b)
50/10	603.1 ± 8.8^b (22114 $\pm$ 68 ^a)	401.4 ± 31.2^c	371.4 ± 6.7^c (9368 $\pm$ 75 ^c)	20.2 ± 5.1^a (379 $\pm$ 33 ^a)	56.7 ± 3.9^c (4068 $\pm$ 10 ^c)
50/20	635.5 ± 13.3^b (31195 $\pm$ 138 ^b)	370.2 ± 23.6^a	328.9 ± 14.8^a (8168 $\pm$ 52 ^a)	31.9 ± 8.4^b (733 $\pm$ 72 ^b)	35.9 ± 2.3^d (2745 $\pm$ 23 ^a)

TABLE 3. Mean apparent digestibility ($\pm$ SD) for the experimental diets. Values in a column not sharing the same superscript were significantly different at $P < 0.05$.

Diet (protein/lipid) (% dry weight)	Digestibility (%)			
	Dry matter	Crude protein	Crude lipid	Gross energy
40/10	64.2 ± 1.3^a	92.6 ± 1.3^a	94.1 ± 1.7^a	74.8 ± 0.9^a
40/20	89.4 ± 1.0^b	95.2 ± 2.6^b	93.7 ± 0.9^a	82.9 ± 2.3^b
50/10	61.8 ± 2.4^a	90.0 ± 3.1^a	88.3 ± 1.5^b	81.0 ± 0.5^b
50/20	87.7 ± 0.9^b	93.4 ± 1.3^{ab}	88.7 ± 1.4^b	90.5 ± 2.2^c

2:1 (v/v) chloroform-methanol (Folch et al. 1957). Ash was determined by heating in a muffle furnace (550°C) to constant weight (16 h; AOAC 1980). Energy was measured by adiabatic oxygen bomb calorimetry. Water content of the diet was determined by freeze drying to a constant weight. Digestibility of the diet was determined by the acid-insoluble method (Atkinson et al. 1984).

The difference between fecal nitrogen loss determined on the basis of digestibility coefficients for protein and nitrogen intake was considered as assimilated nitrogen. Endogenous nitrogen excretion (ENE) is the amount of nitrogen excreted by fish in a resting and postabsorptive phase (Gerking 1971) and was measured in lake trout ($n = 11$; 204 ± 15 g) fasted for 4–12 d. No allowance was made for metabolic fecal nitrogen loss when determining ENE. Exogenous nitrogen was equated to the difference between nitrogen excretion by fasted and nonfasted fish. To estimate energy loss through nonfecal excretion, the weights of the two major excretory products ($\text{NH}_3\text{-N}$ and urea-N) were transformed to energy equivalent units of 24.8 and 23.0 $\text{J} \cdot \text{mg}^{-1}$, respectively (Elliott and Davison 1975).

Experiment 2

Prior to the determination of postprandial patterns of nitrogenous excretion in fed and fasted fish, the two groups of fish were held for 15 d and deprived of food for 2 d as described in experiment 1. The daily pattern of nitrogen excretion was measured for fish ($n = 11$; $190\text{--}223$ g) fasted for 12 d and also for fish fed ($n = 11$; $188\text{--}219$ g) each of the experimental diets at 0.75% live body weight daily for five consecutive days. Water samples were analysed for total N, $\text{NH}_3\text{-N}$, and urea-N at 4-h intervals on the fifth day (10:00–10:00) from chambers containing fed fishes and on the twelfth day of fasting. A preliminary study indicated a stabilization of the nitrogen excretion pattern between the fourth and twelfth day of fasting.

Statistical analyses of the data were made using regression analyses and analysis of variance (ANOVA) with significance accepted at $P < 0.05$ level. Averages were compared with the Tukey's Studentized range test (SAS 1989). A test of significance by Bonferroni's method (SAS 1989) permitted comparison of dietary effects on nitrogenous excretion pattern over 4-h intervals.

Results

The proportion of $\text{NH}_3\text{-N}$ and urea-N excreted ranged from 88.8 to 93.2 and from 4.6 to 8.6% of total N, respectively, for all diets. Dietary protein and lipid concentration interacted to affect total N and $\text{NH}_3\text{-N}$ excretion. High lipid in the diet (20%) caused a reduction in $\text{NH}_3\text{-N}$ excretion ($P < 0.05$; Table 2). Although the urea-N excretion rate was high in fish fed high-lipid diets, it was significant only when dietary protein was also high (50%). Total N excreted and in feces increased ($P < 0.05$) with dietary protein concentration and decreased ($P < 0.05$) with lipid. Body weight of fish ($n = 44$; $171\text{--}269$ g) did not influence daily nitrogen (total N, $\text{NH}_3\text{-N}$, and urea-N) excretion for any of the experimental diets ($P > 0.05$).

The general relationships between nonfecal and fecal energy loss and energy intake (Table 2) are

- (1) $E_n = 78.279 - 0.007 E_i$ ($n = 264$; $R^2 = 0.94$)
- (2) $E_f = 48.276 - 0.004 E_i$ ($n = 44$; $R^2 = 0.79$)

where E_n is nonfecal energy, E_f is fecal energy, and E_i is energy intake, all expressed as joules per kilogram per day.

It is clear from the equations and Table 2 that both fecal and nonfecal energy loss varied inversely with energy intake and lipid concentration at both protein concentrations. Nonfecal $\text{NH}_3\text{-N}$ and fecal nitrogen energy losses were influenced by both dietary protein and lipid concentration ($P < 0.05$). Of

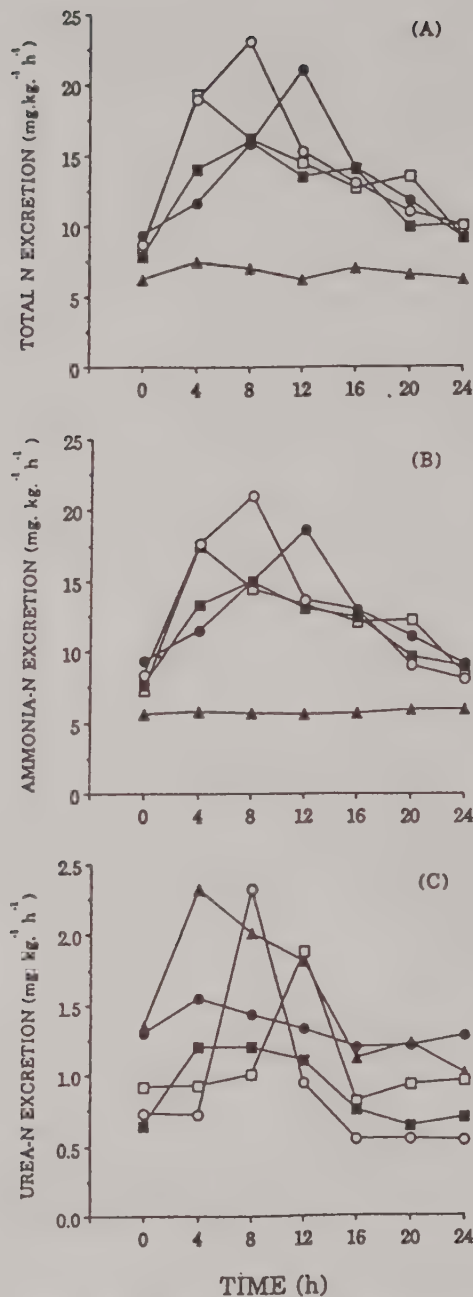


FIG. 1. Daily pattern of nitrogen excretion in lake trout fasted (12 d) and fed test diets (0.75% body wt·d⁻¹) varying in protein and lipid concentrations. Time of feeding is denoted by 0 h. Mean values of (A) total N, (B) ammonia-N, and (C) urea-N are shown for fish fed diet 1 (□) (protein: lipid, 40:10%), diet 2 (■) (40:20%), diet 3 (○) (50:10%), and diet 4 (●) (50:20%) fasted fish (▲).

these, dietary lipid had a greater effect than protein in reducing NH₃-N and fecal energy loss because of larger differences in 95% confidence interval values for the lipid effect (Steel and Torrie 1980). For the low-protein diet, urea energy loss did not differ between the two dietary lipid concentrations ($P > 0.05$). However, for the high-protein diet, high lipid caused a significant urea energy loss.

Digestibility of dry matter and protein increased with lipid ($R^2 = 0.77-0.89$; $P < 0.05$) and decreased ($R^2 = 0.72-0.86$; $P < 0.05$) with protein concentrations (Table 3). Digestibility of lipid was significantly higher for low-protein diets. Energy digestibility increased ($P < 0.05$) with both dietary protein and lipid concentrations. Again, lipid had a greater effect than protein in affecting dietary energy digestibility.

Postprandial rates of total N and NH₃-N excretion in lake trout varied significantly following feeding between the experimental diets (Fig. 1). Urea-N excretion rates fluctuated significantly in fish fed diets 1, 2, and 3. The postprandial variations in total N and NH₃-N excretions for all the experimental diets over 24 h approximated a second-order polynomial equation of the form

$$(3) \quad N = a + b_1 T - b_2 T^2$$

where N is total N or NH₃-N, both expressed as milligrams per kilogram per hour, and T is time in hours.

Prefeeding levels of NH₃-N excretion rates (7.0–9.8 mg·kg⁻¹·h⁻¹) were not different ($P > 0.05$) among the test diets. In low-lipid diets, total N and NH₃-N excretion reached peak values equivalent to 150–175% of prefeeding concentrations between 4 and 8 h following feeding. In high-lipid diets, peak values of total N and NH₃-N excretion were attained between 8 and 12 h, which ranged between only 90–120% of prefeeding levels. Urea-N excretion varied significantly over the 24 h after feeding diets 1, 2, and 3. However, significant differences were not demonstrable in their mean urea-N excretion rates, 0.72, 0.82, and 0.77 mg·kg⁻¹·h⁻¹, respectively. Only diet 4 (50% protein, 20% lipid) resulted in significantly higher urea-N excretion (1.28 mg·kg⁻¹·h⁻¹) compared with the other test diets. In general, high urea-N excretion rates were recorded between 4 and 12 h in fed fish which almost coincided with periods of high NH₃-N excretion rates (Fig. 1). Low-lipid diets had higher ($P < 0.05$) total N and NH₃-N than high-lipid diets 4 h past feeding. By 12 h, excretions of total N and NH₃-N were significantly higher in fish fed diet 4 than the other test diets. In spite of a declining trend, differences ($P > 0.05$) were not demonstrable in postprandial total N and NH₃-N excretion rates between 16 and 24 h among the diets.

In fasted fish, a daily pattern of excretion was found for urea-N but not for total N and NH₃-N ($P > 0.05$). The pattern of urea-N excretion in fish fed diets 1, 2, and 3 and fasted (12 d) over a 24-h period approximated a second-order polynomial equation similar in form to equation (3). The mean total N (8.8 mg·kg⁻¹·h⁻¹) and NH₃-N (6.0 mg·kg⁻¹·h⁻¹) excretion rates in fasted fish were significantly lower than in fed fish (14.1–16.7 and 12.8–15.5 mg·kg⁻¹·h⁻¹, respectively). Urea-N excretion in fasted fish (1.36 mg·kg⁻¹·h⁻¹) was similar ($P > 0.05$) to that for fish fed diet 4 (1.28 mg·kg⁻¹·h⁻¹) and both were significantly higher than those for fish fed diets 1, 2, and 3. Total N and NH₃-N excretions in fasting lake trout (Table 4) decreased ($P < 0.05$) from 260.5 and 225.5 mg·kg⁻¹·d⁻¹, respectively, to 208.3 and 159.9 mg·kg⁻¹·d⁻¹ between 1 and 4 d. With continued fasting to 12 d, total N and NH₃-N excretion remained constant ($P > 0.05$). Urea-N excretion increased ($P < 0.05$) during the initial 4 d of food deprivation from 15.3 to 26.0 mg·kg⁻¹·d⁻¹ as opposed to a significant decrease in NH₃-N excretion. Unlike NH₃-N, urea-N excretion rates progressively increased to become significantly higher (36.5 mg·kg⁻¹·d⁻¹) at 12 d compared with the rates of excretion after 4 d. Body weight (181–226 g) did not have a significant effect on nitrogenous

TABLE 4. Excretion of nitrogen ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) by lake trout during a 12-d fasting period. Each value is expressed on the basis of a 1-kg lake trout and represents the mean $\pm$ SD for 11 fish (204.0 ± 15.0 g). Values in a row not sharing the same superscript were significantly different at $P < 0.05$.

	Food deprivation period (d)			
	1	4	8	12
Total N	260.55 ± 12.12^a	208.27 ± 4.47^b	196.82 ± 6.69^b	194.00 ± 5.06^b
$\text{NH}_3\text{-N}$	225.45 ± 8.77^a	159.91 ± 7.10^b	150.73 ± 20.80^b	139.80 ± 23.29^b
Urea-N	15.28 ± 7.00^a	26.00 ± 11.09^b	33.18 ± 15.06^{bc}	36.45 ± 13.25^c

excretions in fasted fish. Exogenous nitrogen excretion, calculated as the difference in total N of fed and fasted fish, varied from 143.4 to 206.6 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, representing nearly 25% of nitrogen intake in fish fed low-lipid diets when compared with fish fed high-lipid diets (~20%). Similarly, exogenous energy ($\text{NH}_3\text{-N} + \text{urea-N}$) loss varied from 3801.0 to 5347.5 $\text{J} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, being higher in fish fed low-lipid diets at both dietary protein concentrations.

Discussion

The pulses of total N and $\text{NH}_3\text{-N}$ excreted after feeding and the positive relationship between excretion and dietary nitrogen consumption suggest that nitrogenous excretion is primarily of dietary origin in accord with earlier observations on sockeye salmon, *Oncorhynchus nerka* (Brett and Zala 1975). An increase in dietary lipid concentration at specific protein levels reduced $\text{NH}_3\text{-N}$ and total N excretion in lake trout which is consistent with observations made for rainbow trout, *Oncorhynchus mykiss* (previously *Salmo gairdneri*) (Atherton and Aitken 1970), and for common carp, *Cyprinus carpio* (Watanabe et al. 1987). Excretion of urea-N, in contrast, was high in fish fed the high-lipid diet, particularly when dietary protein was also high.

The physiological significance of ureogenesis in freshwater fish that are adapted to excrete ammonia ($\text{NH}_3 + \text{NH}_4^+$) is little understood (Saha and Rath 1987). Due to a constant rate of urea-N excretion under fed and fasted (22 d) conditions in sockeye salmon (Brett and Zala 1975), it was suggested that urea is merely an end product of purine catabolism (Covey 1980). This is supported by the observation that liver arginase activity does not change with dietary arginine concentration in rainbow trout (Walton et al. 1986). In this context, the postprandial fluctuations of urea-N observed in lake trout fed all but one (50% protein, 20% lipid) diet are particularly noteworthy. Increased urea-N excretion and reduced $\text{NH}_3\text{-N}$ excretion in fish fed high-lipid diets probably reflect the involvement of dietary fatty acids in "sparing" the deamination of amino acids (van den Thillard 1986), thus increasing the pool of amino acids for ureogenesis in the liver (Kaushik et al. 1988). M. G. Jayaram and F. W. H. Beamish (unpubl. data) have found that both plasma free amino acids and nonesterified fatty acid concentrations were directly proportional to plasma urea concentrations in lake trout fed diets of different protein and lipid concentrations.

Urea synthesis and excretion in freshwater fish appears to gear towards maintenance of acid-base balance rather than osmoregulation (Randall and Wright 1987). According to Wood et al. (1989), the aminotelic (85% $\text{NH}_3\text{-N}$, 15% urea-N) freshwater teleost, *Oreochromis nilotica* tends to shift towards ureotelism (33% urea-N) as an adaptation for maintaining acid-base balance when exposed to an alkaline (pH ~ 10) environment. Little is known about dietary-induced alkalosis and ureogenesis

in fish. In light of the present observations of a generally close coupling of increased $\text{NH}_3\text{-N}$ excretion with urea-N pulses, it is reasonable to suggest that ureogenesis in lake trout responds to changes in dietary protein and lipid concentrations.

A rapid increase in $\text{NH}_3\text{-N}$ excretion (4–8 h) followed by a precipitous decline to prefeeding levels, as found for fish fed low-lipid diets, is considered inimical for efficient protein utilization (Leid and Braaten 1984). In contrast, maximum rates of $\text{NH}_3\text{-N}$ excretion were achieved slowly (8–12 h) among fish fed high-lipid diets, but once achieved remained at these elevated levels for up to 16 h. A gradual postprandial rise in $\text{NH}_3\text{-N}$ excretion reflects efficient use of protein for fish growth (Dabrowski et al. 1987). Because amino nitrogen cannot be stored in large quantities, a sudden surge of amino acids into the blood leads to increased deamination and nitrogen loss (Covey 1980). The drastic fluctuations in postprandial $\text{NH}_3\text{-N}$ excretion rates in lake trout as opposed to a gradual pulse in urea-N excretion rates suggest a rapid degradation and turnover rate for $\text{NH}_3\text{-N}$ in contrast with a slower turnover rate for urea-N in fish (Watts and Watts 1974).

In fasted lake trout, excretion of urea-N was higher than in fed fish and showed a pattern of diurnal fluctuation. In accord, Guerin-Ancey (1976) reported increased urea-N excretion in bass, *Dicentrarchus labrax*, during a 7-d food deprivation period. The possible cause for higher urea-N excretion during fasting may be linked to increased nonesterified fatty acid concentration in plasma of lake trout (M. G. Jayaram and F. W. H. Beamish, unpubl. data), as found in *D. labrax*, which stimulates hepatic ureogenesis (Zammit and Newshome 1979; Walser 1986). Increased urea-N excretion probably plays a role in cell volume regulation rather than osmoregulation during fasting (Watts and Watts 1974).

Fecal nitrogen loss was less in fish fed high-lipid diets in accord with the results of nutrient digestibility studies which indicate higher digestibility for protein in diets with high lipid concentrations (Cho et al. 1982). Other dietary factors such as a reduction in kaolin (mixture of alumina and silica) and cellulose in high-lipid diets may also have improved digestibility of dietary protein. With an increase in dietary protein, fecal nitrogen loss increased, a pattern also described for other fishes (Savitz 1971; Cho et al. 1982). Although the proportions of protein and lipid in the experimental diets were within the ranges found in natural fish food of salmonids (Phillips et al. 1954), the estimates of energy loss through fecal and nonfecal wastes of lake trout have to be used with caution in determining an energy budget for wild fish. This is necessary, since the percentage of food energy lost in the feces and excretion is greatly influenced by prevailing conditions such as temperature, type and quality of food, and activity level of fish (Elliott 1976; Soofani and Hawkins 1985). Nonetheless, controlled experiments pertaining to the magnitude and composition of nitrogenous losses under different dietary conditions would provide fundamental information about the pattern of dietary protein

utilization (Dabrowski et al. 1987) and nutritional status of fish (Cowey 1980).

In summary, urea-N excretion in lake trout as an alternative end product of protein metabolism which occurs in response to varying dietary protein and lipid concentrations has important implications for feed formulation. This is due to the fact that metabolizable energy is affected by ammonia and urea concentrations in nitrogenous excretion (Cho and Kaushik 1985). On average, a larger proportion of food energy is lost through nitrogenous excretion than in the feces. Besides the effect of dietary lipid on reducing losses of fecal nitrogen and postprandial ammonia-N ($\text{NH}_3 + \text{NH}_4^+$), excretion might aid in keeping ambient ammonia below harmful levels after feeding (McLean and Fraser 1974). Significance of enhanced urea-N excretion during nutritionally dissimilar conditions still needs to be evaluated as a biochemical indicator of protein nutriture in fish.

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References

- ASSOCIATION OF THE OFFICIAL ANALYTICAL CHEMISTS (AOAC). 1980. Official methods of analysis. 13th ed. Association of Official Analytical Chemists, Washington, DC. 1018 p.
- ATHERTON, W. D., AND A. AITKEN. 1970. Growth, nitrogen metabolism and fat metabolism in *Salmo gairdneri*. Comp. Biochem. Physiol. 36: 719-747.
- ATKINSON, J. L., J. W. HILTON, AND S. J. SLINGER. 1984. Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 41: 1384-1386.
- BEAMISH, F. W. H., AND E. THOMAS. 1984. Effects of dietary protein and lipid on nitrogen losses in rainbow trout, *Salmo gairdneri*. Aquaculture 41: 359-371.
- BRETT, J. R., AND T. D. D. GROVES. 1979. Physiological energetics, p. 279-352. In W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. 8. Academic Press, New York, NY.
- BRETT, J. R., AND C. A. ZALA. 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. J. Fish. Res. Board Can. 32: 2479-2486.
- CHO, C. Y., AND S. J. KAUSHIK. 1985. Effects of protein intake on metabolizable and net energy values of fish diets, p. 95-117. In C. B. Cowey, A. M. Mackie, and J. G. Bell [ed.] Nutrition and feeding in fish. Academic Press, New York, NY.
- CHO, C. Y., S. J. SLINGER, AND H. S. BAYLEY. 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B: 25-41.
- COWEY, C. B. 1980. Protein metabolism in fish, p. 271-288. In P. J. Buttery and D. B. Lindsay [ed.] Protein deposition in Animals. Butterworth, London.
- CROCKER, C. L. 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am. J. Physiol. 86: 601-616.
- DABROWSKI, K., S. J. KAUSHIK, AND B. FAUCONNEAU. 1987. Rearing of Baer's sturgeon (*Acipenser baeri* Brandt) larvae. III. Nitrogen and energy metabolism and amino acid absorption. Aquaculture 65: 31-42.
- ELLIOTT, J. M. 1976. Energy losses in the waste products of brown trout (*Salmo trutta* L.). J. Anim. Ecol. 45: 561-580.
- ELLIOTT, J. M., AND W. DAVISON. 1975. Energy equivalents of oxygen consumption in animal energetics. Oecologia 19: 195-201.
- FOLCH, J., M. LEE, AND G. H. S. STANLEY. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.
- GERKING, S. D. 1971. Influence of rate of feeding and body weight on protein metabolism of bluegill sunfish. Physiol. Zool. 44: 9-19.
- GOLDSTEIN, L., AND R. P. FORSTER. 1970. Nitrogen metabolism of fishes, p. 495-517. In Comparative nitrogen metabolism. Vol. 2. Academic Press, New York, NY.
- GUERIN-ANCEY, C. 1976. Experimental study of the nitrogen excretion of bass (*Dicentrarchus labrax*) during growth. 2. Effects of starvation on the excretion of ammonia and urea. Aquaculture 9: 187-194.
- HILTON, J. W., AND S. J. SLINGER. 1981. Nutrition and feeding of rainbow trout. Can. Spec. Publ. Fish. Aquat. 55: 15 p.
- KAUSHIK, S. J., B. FAUCONNEAU, L. TERRIER, AND J. GRAS. 1988. Arginine requirement and status assessed by different biochemical indices in rainbow trout (*Salmo gairdneri* R.). Aquaculture. 70: 75-95.
- LEID, E., AND B. BRAATEN. 1984. The effect of feeding and starving, and different ratios of protein energy to total energy in the feed on the excretion of ammonia in Atlantic cod, *Gadus morhua*. Comp. Biochem. Physiol. 78A: 49-52.
- MCCAULEY, R. L., AND J. S. TAIT. 1970. Preferred temperature of yearling lake trout, *Salvelinus namaycush*. J. Fish. Res. Board Can. 27: 1729-1733.
- MCLEAN, W. E., AND F. J. FRASER. 1974. Ammonia and urea production of coho salmon under hatchery conditions. Environ. Prot. Serv. Pac. Region Surveil. Rep. EPS 5-PR-74-5.
- PHILLIPS, A. M. JR., R. S. NIELSEN, AND D. R. BROCKWAY. 1954. A comparison of hatchery diets and natural food. Prog. Fish-Cult. 16: 153-157.
- POLUNIN, N. V. C., AND I. KOIKE. 1987. Temporal focusing of nitrogen release by a periodically feeding herbivorous reef fish. J. Exp. Mar. Biol. Ecol. 111: 285-296.
- RANDALL, D. J., AND P. A. WRIGHT. 1987. Ammonia distribution and excretion in fish. Fish Physiol. Biochem. 3: 107-120.
- REINITZ, G. L., L. E. ORME, C. A. LEMM, AND F. N. HITZEL. 1978. Influence of varying lipid concentrations with two protein concentrations in diets for rainbow trout (*Salmo gairdneri*). Trans. Am. Fish. Soc. 107: 751-754.
- SAHA, N., AND B. K. RATHA. 1987. Active ureogenesis in freshwater air-breathing teleost, *Heteropneustes fossilis*. J. Exp. Zool. 241: 137-141.
- SAS. 1989. SAS user's guide: statistics. Vol. 2, 1989 ed. SAS Institute Inc., Cary, NC. 584 p.
- SAVITZ, J. 1971. Nitrogen excretion and protein consumption of the bluegill sunfish (*Lepomis macrochirus*). J. Fish. Res. Board Can. 28: 449-451.
- SAYER, M. D. J., AND J. DAVENPORT. 1987. The relative importance of the gills to ammonia and urea excretion in five seawater and one freshwater teleost species. J. Fish Biol. 31: 561-570.
- SLOT, C. 1965. Plasma creatinine determination. A new and specific Jaffe's reaction method. Scand. J. Clin. Lab. Invest. 17: 381-385.
- SOLOROZANO, L. 1969. Determination of ammonia in natural waters by the phenylhypochlorite method. Limnol. Oceanogr. 14: 799-801.
- SOOFANI, N. M., AND A. D. HAWKINS. 1985. Field studies of energy budgets, p. 155-183. In P. Tytler and P. Calow [ed.] Fish energetics, new perspectives. Crom Helm, London.
- STEEL, R. G. D., AND J. H. TORRIE. 1980. Principles and procedures for statistics. McGraw-Hill, New York, NY.
- VAN DEN THILLARD, G. 1986. Energy metabolism of swimming trout (*Salmo gairdneri*). Oxidation rates of palmitate, glucose, lactate, alanine, leucine and glutamate. J. Comp. Physiol. 156: 511-520.
- WALSER, M. 1986. Roles of urea production, ammonium excretion, and amino acid oxidation in acid-base balance. Am. J. Physiol. 250: F181-F188.
- WALTON, M. J., C. B. COWEY, R. M. COLOSO, D. KNOX, AND J. W. ADRON. 1986. Dietary requirement of rainbow trout for tryptophan, lysine and arginine determined by growth and biochemical measurements. Fish Physiol. Biochem. 2: 161-169.
- WATANABE, T., T. TEKEUCHI, S. SATHO, T. IDA, AND M. YAGUCHI. 1987. Development of low protein - high energy diets for practical carp culture with special reference to reduction of total nitrogen excretion. Nippon Suisan Gakkaishi 53: 1413-1423.
- WATTS, R. L., AND D. C. WATTS. 1974. Nitrogen metabolism in fishes, p. 369-446. In M. Florin and B. T. Scheer [ed.] Chemical zoology. Vol. 8. Academic Press, New York, NY.
- WOOD, C. M., S. F. PERRY, P. A. WRIGHT, H. L. BERGMAN, AND D. J. RANDALL. 1989. Ammonia and urea dynamics in lake Magadi tilapia, a ureotelic teleost fish adapted to an extremely alkaline environment. Respir. Physiol. 77: 1-20.
- ZAMMIT, V. A., AND E. A. NEWSHOME. 1979. Activities of enzymes of fat and ketone body metabolism and effects of starvation on blood concentrations of glucose and fat fuels in teleost and elasmobranch fish. Biochem. J. 184: 313-322.

Effect of 6-, 12-, and 18-month Photoperiod Cycles on Smolting and Sexual Maturation in Juvenile Atlantic Salmon (*Salmo salar*)

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The results support the hypothesis that the commencement of smolting can occur during the decreasing phase of the annual photoperiod cycle, and sexual maturation during the increasing phase of the cycle, provided individuals have attained certain (unknown) growth thresholds. Thereafter the completion of smolting is entrained by the increasing phase of the photoperiod cycle, and sexual maturation by the decreasing phase. Three groups of Atlantic salmon (*Salmo salar*) were raised in freshwater for over 2 yr from the eyed egg stage under photoperiod cycles of either 6-, 12- (control), or 18-mo periodicity and an ambient 12-mo temperature cycle. Smolting was judged by changes in salinity tolerance and condition factor. All groups developed bimodal length–frequency distributions by December following hatch. Fish continued to be recruited into the upper modal group (UMG) beyond the shortest day of the photoperiod cycle, providing temperature was not limiting growth. The 6-, 12-, and 18-mo photoperiod cycles resulted in approximately 50, 60, and 100% of the populations being recruited into the UMG. Sexually mature male parr (1+ yr old) occurred only in the lower modal group of the 6- and 12-mo groups.

Les résultats corroborent l'hypothèse selon laquelle le début de la smoltification peut se produire durant la phase descendante du cycle photopériodique annuel et la maturation sexuelle durant la phase ascendante du cycle, à la condition que les individus aient atteint certains seuils de croissance (inconnus). Par la suite, l'achèvement de la smoltification s'effectue durant la phase ascendante du cycle photopériodique et la maturation sexuelle durant la phase descendante. Trois groupes de saumons de l'Atlantique (*Salmo salar*) ont été élevés dans l'eau douce pendant plus de deux ans à partir du stade de l'oeuf embryonné, avec des cycles photopériodiques ayant une périodicité de 6, de 12 (témoin) ou de 18 mo et un cycle de température ambiante de 12 mo. La smoltification a été évaluée par des changements au niveau de la tolérance à la salinité et du coefficient de condition. Dans tous les groupes, on a observé une distribution longueur-fréquence bimodale au mois de décembre suivant l'éclosion. Les poissons ont continué d'être recrutés dans le groupe modal supérieur (UMG) au-delà du jour le plus court du cycle photopériodique lorsque la température ne limitait pas la croissance. Les cycles photopériodiques de 6, 12 et 18 mo ont produit un recrutement de 50, 60 et 100 % des populations dans le groupe modal supérieur. Les tacons mâles ayant atteint la maturité sexuelle (âgés de plus de 1 an) ne se retrouvaient que dans le groupe modal inférieur des groupes de 6 et de 12 mo.

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Atlantic salmon (*Salmo salar*) exhibit flexible life-history patterns, with the age at smolting and sexual maturation being strongly influenced by environmental factors (Saunders et al. 1982; Thorpe 1986; Hoar 1988; Metcalfe and Thorpe 1990). Photoperiod and, to a lesser degree, temperature have important roles in controlling the completion of smolting in spring (Pinder and Eales 1969; Wagner 1974; Jonsson and Ruud-Hansen 1985; Duston and Saunders 1990) and sexual maturation in autumn (Titarev 1975; Nakari et al. 1988; Bromage et al. 1990), but the role of these two environmental factors in the initiation of these processes is less clear.

Smolting and sexual maturation in Atlantic salmon consist of a series of changes extending over several months, the former commencing during autumn and the latter during spring. Both processes are energetically demanding and are undertaken only by individuals that have attained certain growth thresholds, as determined by environmental (Saunders et al. 1982, 1989; Thorpe et al. 1989; Metcalfe and Thorpe 1990), genetic (Bailey et al. 1980; Thorpe et al. 1980), and social factors

(Metcalfe 1991). Thorpe (1986) presented a model relating growth, smolting, and maturation proposing that individual salmon are physiologically aware of their growth rate. Provided this rate is above a genetically determined level during specific "decision periods" in spring and autumn, as defined by the seasonal photoperiod cycle, the fish will commence sexual maturation or smoltification. The model was developed largely from studies on cultured Atlantic salmon that exhibit bimodality in the length–frequency distribution toward the end of the first growing season; the larger, upper mode group (UMG) complete smolting as 1+ -yr-olds and the smaller lower mode group (LMG) at 2+ (Knutsson and Grav 1976; Thorpe 1977, 1989; Bailey et al. 1980; Kristinsson et al. 1985). Under favourable growing conditions, a proportion of fish, mostly males from the LMG, become sexually mature as parr (Bailey et al. 1980; Saunders et al. 1982; Rowe and Thorpe 1990a), which can sometimes inhibit subsequent smolting (Saunders et al. 1982; Lundqvist et al. 1989; Berglund et al. 1991). Similarly, smolting can influence the age at subsequent sexual maturation (Saunders and Henderson 1965; Lundqvist and Fridberg 1982), indicating that developmental routes can be

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affected by preceding life-history events as well as environmental factors.

Support for Thorpe's model comes from experiments in which photoperiod has affected the incidence of smolting and sexual maturation in salmonids (McCormick and Naiman 1984; Duston and Bromage 1988; Adams and Thorpe 1989; Thorpe et al. 1989). Other studies have indicated the important effects of temperature and feeding on growth and its influence on the decision to commence smolting and sexual maturation (Kristinsson et al. 1985; Kazakov et al. 1988; Whitesel 1990; Nicieza et al. 1991; Rowe et al. 1991; Skilbrei 1991). We maintained three groups of Atlantic salmon for over 2 yr under photoperiod cycles with a periodicity of 6, 12 and 18 mo and an ambient 12-mo temperature cycle. The resulting alterations in the incidence and timing of both smolting and sexual maturation revealed further insights into the complex interaction between the seasonal daylength cycle and growth in the control of life-history patterns in Atlantic salmon.

Materials and Methods

Approximately 3000 eyed Atlantic salmon eggs (Saint John River strain) incubated in darkness, except during periodic removal of dead eggs, were delivered to St. Andrews Biological Station on February 17, 1988. Thereafter, about 1000 eggs were maintained in each of three lightproof hatchery troughs and later tanks, under three artificial photoperiod cycles of 6, 12 or 18 mo, all starting at the same daylength (Fig. 1). The photoperiods approximated those of latitude 45°N, ranging from 8 h to 44 min (winter solstice) to 15 h 37 min (summer solstice). There was no twilight period. Illumination at the water

surface was 25 lx from incandescent bulbs up to first feeding and 100 lx from fluorescent light strips thereafter. Photoperiod was controlled by electric timer clocks adjusted manually twice weekly. Fish were supplied with a flow-through supply of aerated freshwater for the entire experiment. Temperature was maintained at 8°C until swim-up (April 7), then increased to 16°C to encourage first feeding, and from July 1988 onwards was ambient (Fig. 1) with an annual cycle ranging between 19°C (August) and 3°C (January–March). Fish were fed daily an excess ration of a commercial dry diet (Corey, Fredericton, N.B.) by automatic feeders providing one feed per hour, adjusted to ensure that the entire daily ration was dispensed during the first 8 h after "lights on."

At approximately monthly intervals, a random sample ($n = 75\text{--}150$) from each group was anaesthetised (1% tertiary amyl alcohol) and measured for fork length (FL; nearest millimetre) and body weight (BW; nearest 0.1 g). Condition factor ($CF = BW \cdot 100/FL^3$) was calculated, and the number of sexually mature males producing milt was recorded. Where the length–frequency distributions were bimodal the length and weight data were subdivided and the mean CF of the LMG and UMG calculated. Estimating the size-break between the LMG and UMG was facilitated by the program Mix (Ichthus Data Systems, Hamilton, Ont.).

Periodically, commencing January 1989, a sample ($n = 5\text{--}10$ fish) was removed from each group and challenged in a 96-h, 37 parts per thousand (ppt) salinity tolerance test (Saunders et al. 1985). If the groups were bimodally distributed, the fish subjected to the test were selected according to body size. Between January and June 1989, only UMG fish were sub-

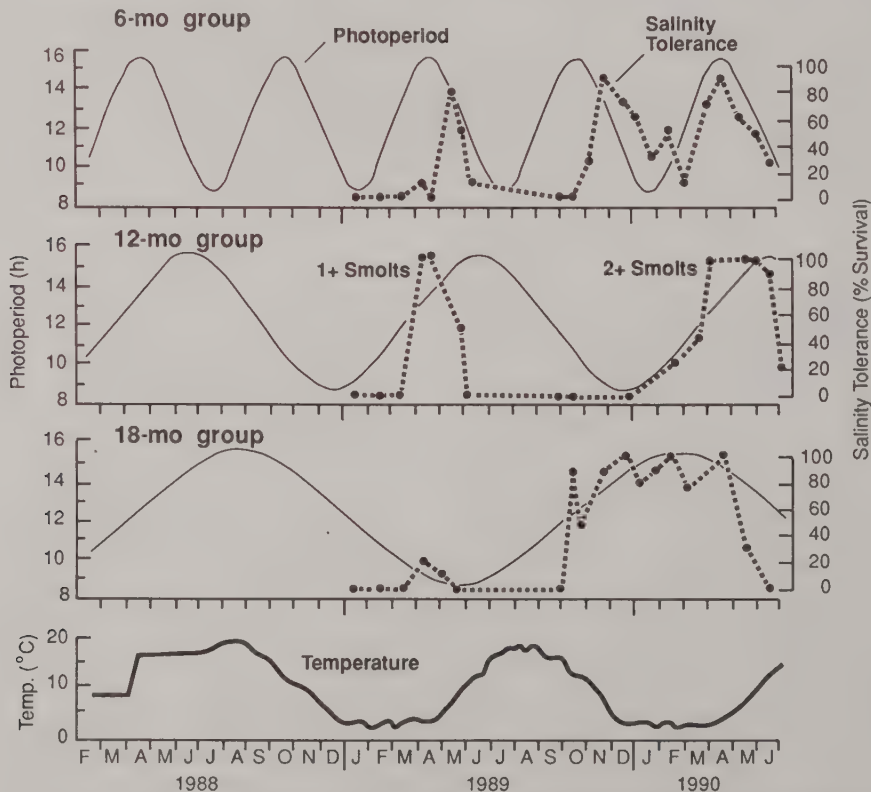


FIG. 1. Experimental photoperiod and temperature regimes and percent survival of Atlantic salmon ($n = 5\text{--}10$) subjected to 96-h, 37-ppt salinity tolerance tests.

jected to salinity tolerance tests. Thereafter, in the 12-mo group, only LMG fish were selected whereas in the 6- and 18-mo groups, bimodality was not evident and a random sample was taken. Fish were directly transferred to a plastic box containing 65 L of static, aerated seawater made up to 37 ppt with Instant Ocean (Aquarium Systems, Mentor, OH) at ambient seawater temperature that ranged from 1.9°C (February) to 13°C (September). At 24-h intervals, dead fish were removed and the number of fish surviving after 96 h expressed as percent survival. Brownlee's test (Brownlee 1960) was used to compare percent survival of fish subjected to salinity tolerance tests. Two-way analysis of variance (ANOVA) followed by one-way ANOVA incorporating Duncan's multiple range test with a significance level set at $P \leq 0.05$ was used to compare means. Mean values are cited with their standard errors (SE).

Results

Length-Frequency Distributions

The length-frequencies of the three groups were unimodally distributed up to early August 1988 (Fig. 2). By September 28, the 6-mo group developed bimodality with an overlap between the two modes at FL of 11–12 cm. By contrast, on the same date the distribution of the 12-mo group was unimodal, but with an extended upper tail, and the 18-mo group appeared normally

distributed. By December 16 the length-frequency distributions of all groups were bimodal, with the upper mode comprising 46% of the population in the 6-mo, 41% in the 12-mo, and 72% in the 18-mo group. The 12-mo group remained bimodally distributed until the end of the experiment in June 1990, with the UMG and LMG having completed smolting as 1+ -yr-olds by May 1989 and 2+ by May 1990. In the 6-mo group the bimodal distribution disappeared during the summer of 1989, with no clear pattern evident from September onwards. In the 18-mo group, following the increase in growth rate in spring 1989, the proportion of fish in the LMG declined. By September 1989, all the 18-mo group had been recruited into the UMG and completed smolting by October 1989.

Condition Factor

All groups exhibited a significant increase in CF following first feeding that peaked in August 1988 and then decreased (Fig. 3). The mean CF of the UMG in all treatments was significantly higher than of the LMG in the same group following the development of bimodality. The UMG of the 12-mo treatment had a significant decrease in mean CF in association with the completion of smolting in May and June 1989 whereas the LMG parr exhibited a significant increase in mean CF over the same period, reaching a peak of 1.34 (0.009) in September

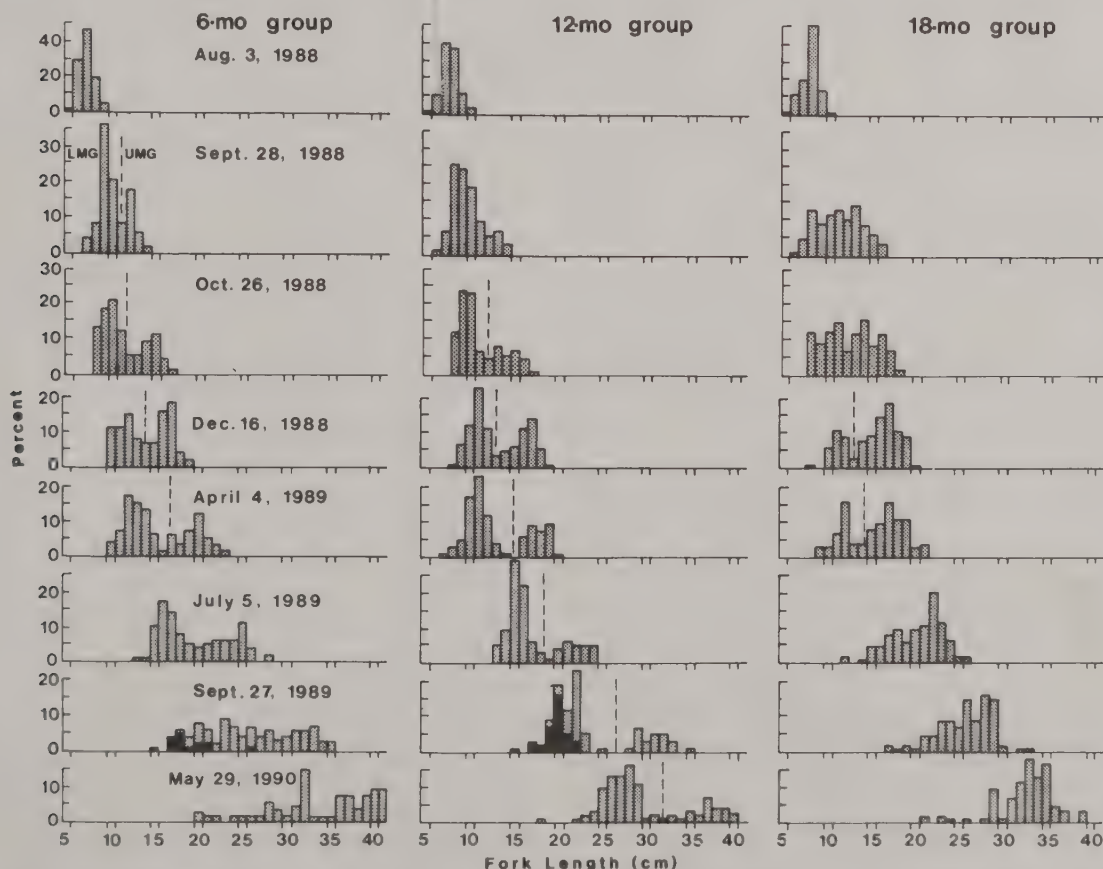


FIG. 2. Length-frequency distributions of Atlantic salmon on dates shown maintained under either 6-, 12-, or 18-mo photoperiod cycles. Solid bars indicate sexually mature males. The vertical broken lines indicate the estimated size-break between the lower (LMG) and upper mode groups (UMG).

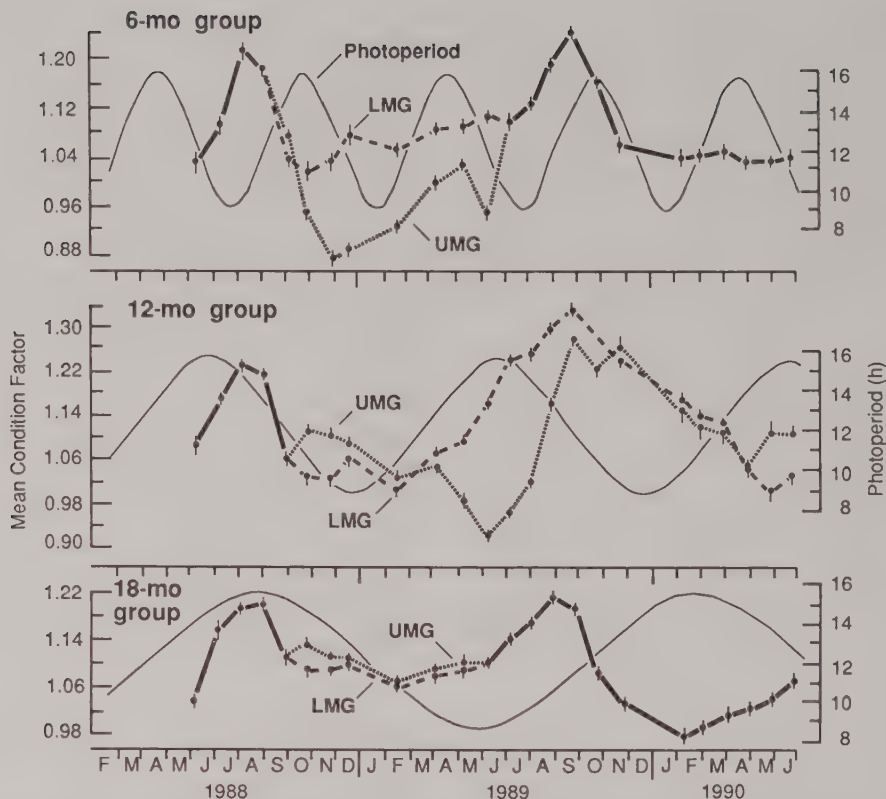


FIG. 3. Effect of 6-, 12-, and 18-mo photoperiod regimes on mean ($\pm$ SE) condition factor. Where bimodality in the distributions was evident, the mean ($\pm$ SE) condition factors of the upper (UMG) and lower modal group (LMG) are plotted; otherwise the coordinates indicate the overall mean of the sample.

1989. The mean CF of the 12-mo UMG increased significantly between July and November 1989, was significantly higher than the 12-mo LMG in November 1989, and was significantly lower during February to early April 1990. The 12-mo LMG completed smolting by May 1990, exhibiting a significantly lower mean CF compared with the UMG postsmolts during May and June 1990. In the 6-mo group, the mean CF of the UMG decreased significantly from 1.08 (0.006) on September 28 to a low of 0.88 (0.013) on December 16, 1988. Over the same period the mean CF of the 6-mo LMG increased and was significantly higher than the UMG from October 1988 until June 1989. From July 1989 onwards, with bimodality no longer evident in the 6-mo group, the total mean CF increased significantly, reaching a peak of 1.25 (0.007) in September 1989, then decreased to 1.07 (0.011) in November, and exhibited no significant change thereafter. In the 18-mo group, the mean CF of the UMG was significantly higher than of the LMG from October 1988 until the disappearance of bimodality by June 1989, but during this period, both modes exhibited similar changes in mean CF with respect to time. The overall mean CF in the 18-mo group increased significantly through 1989, reaching a peak of 1.21 (0.011) in August, and then decreased significantly in association with the completion of smolting to a low of 0.98 (0.010) in February 1990.

Salinity Tolerance

Salinity tolerance in the 12-mo UMG significantly increased between March and April 1989 and declined during May to 0%

survival in early June (Fig. 1). The following year, the 12-mo LMG showed a significant increase in salinity tolerance from 0% survival in January to 25% survival in February, exhibiting 100% survival between early April and June, declining significantly thereafter, exhibiting 20% survival in early July 1990. The 6-mo UMG exhibited an increase in salinity tolerance during 1989 that was significantly delayed compared with the 12-mo group, increasing from 0% survival in late April to a peak of 80% survival in mid-May. Salinity tolerance then declined again to 10% survival in a test starting mid-June 1989. A second increase in salinity tolerance in the 6-mo group occurred in late 1989, increasing significantly from 0% survival in mid-October to 90% survival in late November. All fish surviving the tolerance tests during this period had FL $\geq$ 22.8 cm. Salinity tolerance in the 6-mo group then declined gradually, reaching a low in early March 1990 and then increasing to a third peak of 90% survival in late April, thereafter declining to 30% survival in late June. The 18-mo group showed a small but significant increase in salinity tolerance in spring 1989, exhibiting 20% survival in mid-April. Salinity tolerance in the 18-mo group increased significantly during October 1989, exhibiting 70–100% survival between November 1989 and April 1990. Salinity tolerance decreased significantly from 100% survival in late April to 30% survival in late May and 0% survival in a final test in mid-June 1990.

Sexual Maturation

Sexually mature males were found in the 6-mo group from August 30 and in the 12-mo group from September 27, 1989,

until the following April (Fig. 2). The proportion of sexually mature males in the 6-mo group reached a maximum of 27% in October 1989, declining to 17% in February, 15% in March, and 8% in April. The proportion of sexually mature males in the 12-mo group ranged from 32 to 37% between September 1989 and March 1990, declining to 26% in April 1990. Sexually mature males were amongst the smallest fish in the 6- and 12-mo groups. In the 18-mo group, apart from two sexually mature males in October 1989 (FL 18.6 and 20.2 cm) and one in February 1990 (FL 24.9 cm), the fish were immature throughout the experiment.

Discussion

Maintaining juvenile Atlantic salmon under 6-, 12-, and 18-mo photoperiods affected growth dynamics and also the timing and incidence of gonadal maturation and salinity tolerance. The results support the hypothesis that the decreasing (or increasing) phase of the annual daylength cycle defines periods during which individual fish can make the physiological decision to commence smolting (or sexual maturation). The development of bimodality in the length–frequency distributions and the recruitment of fish into the UMG can extend for several months from around the summer solstice until beyond the winter solstice, providing temperature is high enough to allow growth. Thereafter the increasing phase of the daylength serves as a Zeitgeber entraining the completion of smolting. The annual photoperiod cycle appears to play a similar role in the control of sexual maturation, except that the completion of sexual maturation is entrained by the phase of decreasing daylength. Other studies suggest that the decision to commence maturation occurs after the winter solstice when the daylength is increasing.

The commencement of smolting is a cause, or an effect, of an increase in somatic growth rate that can be observed from September (Kristinsson et al. 1985; Metcalfe et al. 1988; Wright et al. 1990) until the end of the growing season (Kristinsson et al. 1985). The body size at which individuals are recruited into the UMG is not fixed, but increases progressively as the mean body size increases (Kristinsson et al. 1985). Bimodality was evident first in the 6-mo group in late September, then in the 12-mo group, and last in the 18-mo group in December (Fig. 1), confirming that the phase of the photoperiod cycle has an influence on the establishment of the two size modes (Villareal et al. 1988; Thorpe et al. 1989; Saunders et al. 1989; Skilbrei 1991). An extended photoperiod after midsummer prolongs entry into the UMG, resulting in a delay in the development of bimodality (Fig. 1) (Saunders et al. 1989; Skilbrei 1991), possibly due to the continued recruitment of fish into the UMG as a result of the direct stimulatory effect of a long photoperiod on growth rate (Saunders and Harmon 1990; Stewart et al. 1990). Although the seasonal daylength cycle is important in the control of the growth dynamics (Fig. 2, 3), a decrease in daylength following midsummer is not essential for the development of bimodality, as parr maintained on a constant long photoperiod from June 21 onwards separated into two size modes (J. Duston and R. L. Saunders, unpubl. obs.). This seemingly indirect role of photoperiod in the control of growth patterns is analogous to previous studies on spawning in salmonids (see Duston and Bromage 1991) and suggests that seasonal cycle of daylength serves as a Zeitgeber, influencing the timing of development of bimodality through the entrainment of an endogenous circannual rhythm or clock.

The termination of the decision period to commence smolting appears to be entrained by the increasing phase of the pho-

toperiod cycle. Following the resumption of growth in spring 1989, the 12-mo group exhibited no further recruitment into the UMG whereas in the 18-mo group recruitment into the UMG resumed. It is proposed that this continued recruitment in the 18-mo group was due to the photoperiod being at the winter phase of the cycle, resulting in an extension of the smolt decision period. By contrast, in the 12-mo group the increasing phase of the photoperiod cycle served the dual function of terminating the smolt decision period and also entraining the completion of smolting in those fish already recruited into the UMG. Following this hypothesis, the 6-mo group received a smolt decision period every 6 mo, which may explain the change in the length–frequency distribution (Fig. 2) from unimodal (August 1988), through bimodal (April 1989), to a broader, flatter distribution (September 1989), possibly the result of development of a third size mode. Overall, the results disagree with Thorpe (1986) who proposed that the duration of the decision period for smolting lasts only about 1 mo. The present and previous studies indicate that the decision period to commence smolting appears to extend at least until the winter solstice, providing temperature is not limiting growth (Kristinsson et al. 1985; Kazakov et al. 1988; Whitesel 1990; Nicieza et al. 1991). A possible explanation for this disagreement is that Thorpe's model was developed from studies at Almondbank hatchery in Scotland (latitude 56°N) where water temperatures from midsummer until December are lower than at St. Andrews, N.B. (Fig. 1) (see Thorpe et al. 1989; Rowe and Thorpe 1990a) and might account for the shorter period for recruitment into the UMG.

Following recruitment into the UMG, the increasing phase of the photoperiod cycle entrains the completion of smolting. The UMG's of the 6- and 12-mo treatments exhibited large decreases in mean CF during October–November 1988 and April–June 1989, respectively, in association with the increasing daylength (Fig. 3). By contrast, over the same period the LMG parr of the 6- and 12-mo groups showed only a small change, or significant increase in CF, emphasising the divergence in life-history pattern between the LMG and UMG. A temporary decrease in CF is a characteristic of the completion of smolting and is at least partly due to catabolism of body energy reserves and an elongation in body shape (Farmer et al. 1978). In the 18-mo group during early 1989, the UMG and LMG exhibited similar changes in CF, indicating that both modes were exhibiting similar patterns of growth as a result of the decreasing photoperiod. Thereafter in the 18-mo group the increasing phase of the daylength from June 1989 onwards resulted in the completion of smolting in October 1989, which was associated with a large decrease in CF. Finally, in spring 1990 the LMG of the 12-mo group completed smolting (2+ smolts) and exhibited a decrease in CF similar to that observed in the UMG when they completed smolting in 1989 (1+ smolts). In spring 1990 the 12-mo UMG postsmolts did not exhibit a decrease in CF, supporting the hypothesis that the decrease in CF and other morphological (Gorbman et al. 1982) and growth changes (Chernitskiy and Loenko 1983) associated with smolting are once-in-a-lifetime events. In contrast, hypoosmoregulatory ability in postsmolts retained in freshwater appears to exhibit a seasonal cycle (Hoar 1965; Staggs et al. 1989).

The results of the salinity tolerance tests confirm that the increasing phase of the photoperiod cycle has an important role in the development of this aspect of smolting (see Duston and Saunders 1990). The 12-mo group exhibited two periods of increased salinity tolerance as the UMG completed smolting

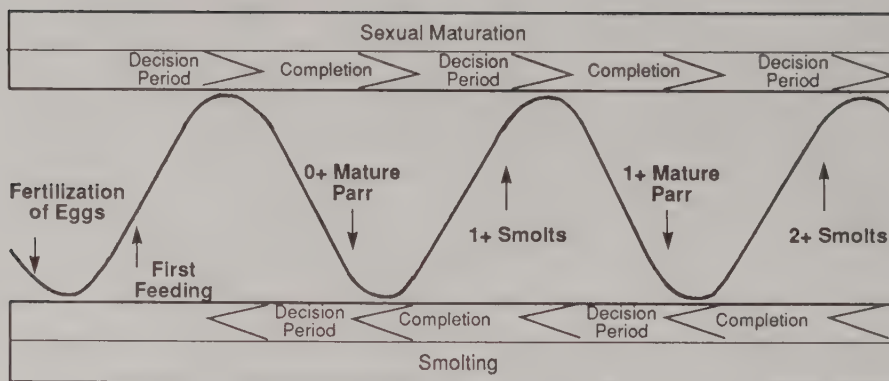


FIG. 4. Model indicating the role of the seasonal cycle in daylength in the control of smolting and sexual maturation in Atlantic salmon. The increasing and decreasing phases of the photoperiod cycle define decision periods during which fish can commence maturation and smolting, respectively, providing they have attained certain (unknown) energy reserve/size thresholds. Thereafter, the completion of smolting and maturation is entrained by the increasing and decreasing phases of the photoperiod cycle. The figure indicates the uncertainty as to the duration of the decision periods.

during March–April 1989 and the LMG in 1990 (Fig. 1). Similarly, the 18-mo group exhibited high salinity tolerance from October 1989 until the following May, during the period of increasing daylength. In contrast, the increasing phase of the photoperiod cycle was not associated with an increase in salinity tolerance in the UMG of the 6-mo group during October–December 1988, despite the decrease in CF, supporting the finding that these two changes associated with smolting can be dissociated (Björnsson et al. 1989; Duston and Saunders 1990). Thereafter, the 6-mo group exhibited three periods of high salinity tolerance, occurring in association with the increasing photoperiod in early 1989, late 1989, and early 1990.

Overall, the results from the three groups support the hypothesis that the seasonal change in daylength serves to entrain an endogenous circannual rhythm of salinity tolerance (Eriksson and Lundqvist 1982; Duston and Saunders 1990). The shorter the periodicity of the photoperiod cycle, the more progressively phase-delayed is the period of high salinity tolerance, as predicted by entrainment theory (Aschoff 1980; Gwinner 1986) and observed in other studies on the entrainment of circannual rhythms in salmonids (Bromage and Duston 1986; Thrush and Bromage 1988), a bird (Gwinner 1977), and a mammal (Goss et al. 1974). Two observations inconsistent with the hypothesis are that the 18-mo group exhibited a small increase in salinity tolerance in April–May 1989 when the photoperiod was short and that in the 6-mo group the third increase in salinity tolerance in March–May 1990 was phase-advanced compared with the photocycle instead of phase-delayed as predicted by entrainment theory. Both these increases in salinity tolerance occurred in spring in association with an increase in somatic growth rate. It is possible that increases in temperature stimulate the development of hypoosmoregulatory mechanisms independently of photoperiod through the stimulation of growth and associated increases in circulating growth hormone levels (Collie et al. 1989; Madsen 1990). Further increases in temperature above 10°C cause a reduction in gill Na^+ , K^+ -ATPase activity and smolt characteristics (see Duston et al. 1991) and account, in part, for the decreases in salinity tolerance in all groups in late May. However, decreases in salinity tolerance also occurred in the 6-mo group at <10°C during December 1989 to February 1990 and in the 18-mo group during March 1990, indicating

that temperature is not solely responsible for decreases in salinity tolerance. This result supports the hypothesis of an underlying circannual rhythm of salinity tolerance entrained by photoperiod (see Duston and Saunders 1990).

The seasonal photoperiod cycle appears to have a similar Zeitgeber role in the completion of sexual maturation as it does in the control of smolting, except that the timing of events are shifted in phase by about 6 mo. The decision period to commence sexual maturation in salmonids occurs during the increasing phase of the photoperiod cycle, as indicated by histological, endocrine (Sumpter et al. 1984; Bromage and Cumaratunga 1988), feeding (Adams and Thorpe 1989; Rowe and Thorpe 1990b), and photoperiod studies (McCormick and Naiman 1984; Duston and Bromage 1988; Adams and Thorpe 1989). The proportion of the population commencing sexual maturation during this decision period has been correlated with condition factor (Rowe and Thorpe 1990a), fat levels (Rowe et al. 1991), and opportunity for growth (Adams and Thorpe 1989; Rowe and Thorpe 1990b). None of the groups in our study produced mature fish in response to the decision period defined by the increasing daylength in 1988, presumably because these underyearlings failed to satisfy the energy reserve requirements for maturation. Only under exceptional growing conditions do Atlantic salmon mature as underyearlings, and then only males (Bailey et al. 1980; Saunders et al. 1982). It is proposed that the subsequent phase of increasing daylength in the 6- and 12-mo groups between January–April and January–June 1989, respectively, offered the fish another decision period for maturation. During this decision period, a proportion of LMG male parr in both the 6- and 12-mo groups satisfied the requirements for maturation and commenced gonadal development. Thereafter, the completion of maturation was entrained by the phase of decreasing daylength, occurring in September 1989 in the 12-mo group, and advanced to late August in the 6-mo group, similar to previous studies (review: Bromage et al. 1990). The size distribution of the mature males in the 6- and 12-mo treatments (Fig. 2) indicates that only LMG parr matured, and UMG postsmolts did not mature, supporting the finding that completion of smolting inhibits sexual maturation that same season (Lundqvist and Fridberg 1982; Thorpe 1986). This inhibition may be the result of depletion of energy reserves

during smolting, as under favourable growing conditions, Atlantic salmon can mature as postsmolts (Saunders and Henderson 1965; Suterlin et al. 1978; Lundqvist and Fridberg 1982; Eriksson et al. 1987). The 18-mo photoperiod cycle resulted in virtually no sexually mature males. None of the 18-mo fish satisfied the requirements for sexual maturation as underyearlings, despite the extended period of increasing daylength offering a longer decision period for maturation. Thereafter, it is proposed that the phase of decreasing daylength from September 1988 to May 1989 ended the decision period for maturation and started the decision period for smoltification. As described above, the majority of the 18-mo group completed smolting in late 1989 as the result of all the fish having satisfied the energetic requirements for smolting during this extended decision period.

In conclusion, the results indicate the important role of the annual cycle of daylength in the control of the life-history patterns in juvenile Atlantic salmon. Parr commence smolting during a "smolt decision period" defined by the phase of decreasing daylength, providing they have achieved sufficient growth, with the completion of smolting entrained by the increasing daylength in spring. The phase of increasing daylength also defines a "maturation decision period" during which fish can commence sexual maturation, again, providing they have attained sufficient growth "thresholds" (Fig. 4). The duration of the decision periods remains unknown, but it is clear that the smolt decision period can continue much longer than proposed by Thorpe's (1986) model. Although smolting and sexual maturation can be mutually inhibitory (Thorpe 1986), one does not preclude the other under conditions favourable to growth. Therefore, it is proposed that smolting and sexual maturation are only indirectly inhibitory of each other through the energetic cost of each process. Although it is apparent that the "thresholds" for smolting and maturation are associated with growth and accumulation of energy reserves (Thorpe 1986), attempts to determine the causal effects have indicated that they are complex and difficult to define (Wright et al. 1990; Metcalfe 1991; Rowe et al. 1991).

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References

- ADAMS, C. A., AND J. E. THORPE. 1989. Photoperiod and temperature effects on early development and reproductive investment in Atlantic salmon (*Salmo salar* L.). *Aquaculture* 79: 403–409.
- ASCHOFF, J. 1980. Biological clocks in birds, p. 113–136. *In* Proc. 17th Int. Ornithol. Congr., Berlin.
- BAILEY, J. K., R. L. SAUNDERS, AND M. I. BUZETA. 1980. Influence of parental smolt age and sea age on growth and smolting of hatchery-reared Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 37: 1379–1386.
- BERGLUND, I., L. P. HANSEN, H. LUNDQVIST, B. JONSSON, T. ERIKSSON, J. E. THORPE, AND L. O. ERIKSSON. 1991. Effects of elevated winter temperature on seawater adaptability, sexual rematuration, and downstream migratory behaviour in mature male Atlantic salmon parr (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 48: 1041–1047.
- BJORNSSON, B. T., H. THORARENSEN, T. HIRANO, T. OGASAWARA, AND J. B. KRISTINSSON. 1989. Photoperiod and temperature affect plasma growth hormone levels, growth, condition factor and hypothyroidism ability of juvenile Atlantic salmon (*Salmo salar*) during parr-smolt transformation. *Aquaculture* 82: 77–91.
- BROMAGE, N., AND R. CUMARANATUNGA. 1988. Egg production in the rainbow trout, p. 63–138. *In* J. F. Muir and R. J. Roberts [ed.] Recent advances in aquaculture. Vol. 3. Croom Helm, London and Sydney.
- BROMAGE, N., AND J. DUSTON. 1986. The control of spawning in the rainbow trout (*Salmo gairdneri* Richardson) using photoperiod techniques. *Inst. Freshwater Res. Drottningholm Rep.* 63: 26–35.
- BROMAGE, N., J. DUSTON, C. RANDALL, A. BROOK, M. THRUSH, M. CARILLO, AND S. ZANUY. 1990. Photoperiodic control of teleost reproduction, p. 620–626. *In* A. Eppele, C. G. Scanes, and M. H. Stetson [ed.] Proc. 11th Int. Symp. Comp. Endocrinol., Malaga, Spain.
- BROWNLEE, K. A. 1960. Statistical theory and methodology of science and engineering. J. Wiley and Sons, Inc., New York, NY. 570 p.
- CHERNITSKIY, A. G., AND A. A. LOENKO. 1983. The osmoregulatory system and possible ways of differentiation in ecological forms of Atlantic salmon, *Salmo salar*. *J. Ichthyol.* 23: 84–94.
- COLLIE, N. L., J. P. BOLTON, H. KAWAUCHI, AND T. HIRANO. 1989. Survival of salmonids in seawater and the time frame of growth hormone action. *Fish Physiol. Biochem.* 7: 315–321.
- DUSTON, J., AND N. BROMAGE. 1988. The entrainment and gating of the endogenous circannual rhythm of reproduction in the female rainbow trout (*Salmo gairdneri*). *J. Comp. Physiol. A* 164: 259–268.
1991. Circannual rhythms of gonadal maturation in female rainbow trout (*Oncorhynchus mykiss*). *J. Biol. Rhythms* 6: 49–53.
- DUSTON, J., AND R. L. SAUNDERS. 1990. The entrainment role of photoperiod on hypothyroidism and growth related aspects of smolting in Atlantic salmon (*S. salar*). *Can. J. Zool.* 68: 707–715.
- DUSTON, J., R. L. SAUNDERS, AND D. E. KNOX. 1991. Effects of increases in freshwater temperature on loss of smolt characteristics in Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 48: 164–169.
- ERIKSSON, L. O., AND H. LUNDQVIST. 1982. Circannual rhythms and photoperiod regulation of growth and smolting in Baltic salmon (*Salmo salar* L.). *Aquaculture* 28: 113–121.
- ERIKSSON, T., L. O. ERIKSSON, AND H. LUNDQVIST. 1987. Adaptive flexibility in life history tactics of mature male Baltic salmon parr in relation to body size and environment. *Am. Fish. Soc. Symp.* 1: 236–243.
- FARMER, G. J., J. A. RITTER, AND D. ASHFIELD. 1978. Seawater adaptation and parr-smolt transformation of juvenile Atlantic salmon, *Salmo salar*. *J. Fish. Res. Board Can.* 35: 93–100.
- GORBMAN, A., W. W. DICKHOFF, J. L. MIGHELL, E. F. PRENTICE, AND F. W. WAKNITZ. 1982. Morphological indices of developmental progress in the parr-smolt coho salmon, *Oncorhynchus kisutch*. *Aquaculture* 28: 1–19.
- GOSS, R. J., C. E. DINSMORE, L. N. GRIMES, AND J. K. ROSEN. 1974. Expression and suppression of the circannual antler growth cycle in deer, p. 393–422. *In* E. T. Pongelley [ed.] Circannual clocks. Academic Press, New York, NY.
- GWINNER, E. 1977. Photoperiodic synchronisation of circannual rhythms in the European starling (*Sturnus vulgaris*). *Naturwissenschaften* 64: 44–45.
1986. Circannual rhythms. *Zoophysiology*. Vol. 18. Springer, Berlin. 154 p.
- HOAR, W. S. 1965. The endocrine system as a chemical link between the organism and its environment. *Trans. R. Soc. Can. Ser. IV* 3: 175–200.
1988. The physiology of smolting salmonids, p. 275–343. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. 11B. Academic Press, New York, NY.
- JONSSON, B., AND J. RUUD-HANSEN. 1985. Water temperature as the primary influence on timing of seaward migration of Atlantic salmon (*Salmo salar*) smolts. *Can. J. Fish. Aquat. Sci.* 42: 593–595.
- KAZAKOV, R. V., O. L. CHRISTOFOROV, I. G. MURZA, S. A. ILYENKOVA, AND S. F. TITOV. 1988. Results of accelerated rearing of Atlantic salmon, *Salmo salar* L., smolts by use of warm waste water. *J. Fish Biol.* 32: 869–880.
- KNUTSSON, S., AND T. GRAY. 1976. Seawater adaptation in Atlantic salmon (*Salmo salar* L.) at different experimental temperatures and photoperiods. *Aquaculture* 8: 169–187.
- KRISTINSSON, J. B., R. L. SAUNDERS, AND A. J. WIGGS. 1985. Growth dynamics during the development of bimodal length-frequency distribution in juvenile Atlantic salmon (*Salmo salar* L.). *Aquaculture* 45: 1–20.
- LUNDQVIST, H., B. BORG, AND I. BERGLUND. 1989. Androgens impair seawater adaptability in smolting Baltic salmon (*Salmo salar*). *Can. J. Zool.* 67: 1733–1736.
- LUNDQVIST, H., AND G. FRIDBERG. 1982. Sexual maturation versus immaturity: different tactics with adaptive values in Baltic salmon (*Salmo salar*) male smolts. *Can. J. Zool.* 60: 1822–1827.
- MADSEN, S. S. 1990. The role of cortisol and growth hormone in seawater adaptation and development of hypothyroidism mechanisms in sea trout parr (*Salmo trutta trutta*). *Gen. Comp. Endocrinol.* 79: 1–11.
- MCCORMICK, S. D., AND R. J. NAIMAN. 1984. Some determinants of maturation in brook trout, *Salvelinus fontinalis*. *Aquaculture* 43: 269–278.
- METCALFE, N. B. 1991. Competitive ability influences seaward migration age in Atlantic salmon. *Can. J. Zool.* 69: 815–817.

- METCALFE, N. B., F. A. HUNTINGFORD, AND J. E. THORPE. 1988. Feeding intensity, growth rates, and the establishment of life-history patterns in juvenile Atlantic salmon, *Salmo salar*. J. Anim. Ecol. 57: 463–474.
- METCALFE, N. B., AND J. E. THORPE. 1990. Determinants of geographical variation in the age of seaward-migrating salmon, *Salmo salar*. J. Anim. Ecol. 59: 135–145.
- NAKARI, T., A. SOIVIO, AND S. PESONEN. 1988. Effects of an advanced photoperiod cycle on the gonadal development and spawning time of 2 year-old *Salmo gairdneri* R. reared in earth ponds under extreme annual water temperatures. Aquaculture 67: 369–384.
- NICIEZA, A. G., F. BRANA, AND M. M. TOLEDO. 1991. Development of length-bimodality in wild stocks of Atlantic salmon, *Salmo salar* L., under different growth conditions. J. Fish Biol. 38: 509–523.
- PINDER, L. J., AND J. G. EALES. 1969. Seasonal buoyancy changes in Atlantic salmon (*Salmo salar*) parr and smolts. J. Fish. Res. Board Can. 26: 2093–2100.
- ROWE, D. K., AND J. E. THORPE. 1990a. Differences in growth between maturing and non-maturing male Atlantic salmon (*Salmo salar* L.) parr. J. Fish Biol. 36: 643–658.
- 1990b. Suppression of maturation in male Atlantic salmon (*Salmo salar* L.) parr by reduction in feeding and growth during spring months. Aquaculture 86: 291–313.
- ROWE, D. K., J. E. THORPE, AND A. M. SHANKS. 1991. Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. Can. J. Fish. Aquat. Sci. 48: 405–413.
- SAUNDERS, R. L., AND P. R. HARMON. 1990. Influence of photoperiod on growth of juvenile Atlantic salmon and development of salinity tolerance during winter–spring. Trans. Am. Fish. Soc. 119: 689–697.
- SAUNDERS, R. L., AND E. B. HENDERSON. 1965. Precocious sexual development in male postsmolt Atlantic salmon reared in the laboratory. J. Fish. Res. Board Can. 22: 1567–1570.
- SAUNDERS, R. L., E. B. HENDERSON, AND B. D. GLEBE. 1982. Precocious sexual maturation and smoltification in male Atlantic salmon (*Salmo salar*). Aquaculture 28: 211–229.
- SAUNDERS, R. L., E. B. HENDERSON, AND P. R. HARMON. 1985. Effects of photoperiod on juvenile growth and smolting of Atlantic salmon and subsequent survival and growth in sea cages. Aquaculture 45: 55–66.
- SAUNDERS, R. L., J. L. SPECKER, AND M. P. KOMOURDJIAN. 1989. Effects of photoperiod on growth and smolting in juvenile Atlantic salmon (*Salmo salar*). Aquaculture 82: 103–117.
- SKILBREI, O. T. 1991. Importance of threshold length and photoperiod for the development of bimodal length–frequency distribution in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 48: 2163–2172.
- STAGG, R. M., C. TALBOT, F. B. EDDY, AND M. WILLIAMS. Seasonal variations in osmoregulatory and respiratory responses to seawater exposure of juvenile Atlantic salmon (*Salmo salar*) maintained in freshwater. Aquaculture 82: 219–228.
- STEWART, M. W., R. L. SAUNDERS, AND A. J. WIGGS. 1990. Effects of extended daylength on autumn growth dynamics of juvenile Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. 47: 755–759.
- SUMPTER, J. P., A. P. SCOTT, S. M. BAYNES, AND P. R. WITTHAMES. 1984. Early stages of the reproductive cycle in virgin female rainbow trout (*Salmo gairdneri*). Aquaculture 43: 235–242.
- SUTTERLIN, A. M., P. HARMON, AND B. YOUNG. 1978. Precocious sexual maturation in Atlantic salmon (*Salmo salar*) postsmolts reared in a seawater impoundment. J. Fish. Res. Board Can. 35: 1269–1272.
- THORPE, J. E. 1977. Bimodal distribution of length of juvenile Atlantic salmon (*Salmo salar* L.) under artificial rearing conditions. J. Fish. Biol. 11: 175–184.
1986. Age at first maturity in Atlantic salmon, *Salmo salar*: freshwater period influences and conflicts with smolting, p. 7–14. In D. J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.
1989. Developmental variation in salmonid populations. J. Fish Biol. 35(Suppl. A): 295–303.
- THORPE, J. E., C. E. ADAMS, M. S. MILES, AND D. S. KEAY. 1989. Some influences of photoperiod and temperature on opportunity for growth in juvenile Atlantic salmon, *Salmo salar* L. Aquaculture 82: 119–126.
- THORPE, J. E., R. I. G. MORGAN, E. M. OTTAWAY, AND M. S. MILES. 1980. Time of divergence of growth groups between potential 1+ and 2+ smolts among sibling Atlantic salmon. J. Fish Biol. 17: 13–21.
- THRUSH, M. A., AND N. R. BROMAGE. 1988. Photoperiodic effects on smoltification in Atlantic salmon (*Salmo salar*). J. Int. Disp. Cycle Res. 19: 213.
- TITAREV, YE. F. 1975. Acceleration of maturation in the rainbow trout (*Salmo gairdneri*) under influence of increased water temperature. J. Ichthyol. 15: 507–509.
- VILLAREAL, C. A., J. E. THORPE, AND M. S. MILES. 1988. Influence of photoperiod on growth changes in juvenile Atlantic salmon, *Salmo salar* L. J. Fish Biol. 33: 15–30.
- WAGNER, H. H. 1974. Photoperiod and temperature regulation of smolting in steelhead trout (*Salmo gairdneri*). Can. J. Zool. 52: 219–234.
- WHITESEL, T. A. 1990. Performance of juvenile Atlantic salmon (*S. salar*) introduced into a stream: smolt development and thyroid hormones. Ph.D. thesis, University of Rhode Island, Kingston, RI. 140 p.
- WRIGHT, P. J., N. B. METCALFE, AND J. E. THORPE. 1990. Otolith and somatic growth rates in Atlantic salmon parr (*Salmo salar* L.): evidence against coupling. J. Fish Biol. 36: 241–249.

Light, Nutrients, and Water Temperature as Determinants of Phytoplankton Production in Two Saline, Prairie Lakes with High Sulphate Concentrations

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Our data support empirical models indicating that algal productivity is low relative to total phosphorus (TP) levels in prairie lakes with high sulphate concentrations. Mean chlorophyll accounted for 91.1% of the variance in euphotic zone primary production (ΣA) in Humboldt Lake (total dissolved solids (TDS) = $3.3 \text{ g}\cdot\text{L}^{-1}$; $Z_{\text{max}} = 6 \text{ m}$), while TP, total dissolved phosphorus, and water temperature accounted for 82.7% of ΣA variance in Redberry Lake (TDS = $20.9 \text{ g}\cdot\text{L}^{-1}$; $Z_{\text{max}} = 17 \text{ m}$). The relative importance of these variables to ΣA resulted from biological, chemical, and physical differences of these lakes. Light usually penetrated to the bottom of Redberry Lake due to a mean euphotic zone (Z_{eu}) chlorophyll of $1.7 \text{ mg}\cdot\text{m}^{-3}$, while Humboldt Lake's mean Z_{eu} was 3.4 m with a mean chlorophyll concentration of $62.6 \text{ mg}\cdot\text{m}^{-3}$. Chlorophyll was the dominant factor correlated with light penetration in Humboldt Lake ($r^2 = 0.65$) but not in Redberry Lake. Photosynthetic capacity was correlated ($r^2 = 0.72$) with water temperature only in Redberry Lake. The mean ΣA was 57.1 and $230.2 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ for Redberry and Humboldt lakes, respectively.

Nos données corroborent les modèles empiriques indiquant que la productivité des algues est faible par rapport aux teneurs en phosphore totale (PT) dans les lacs des Prairies ayant une concentration de sulfate élevée. La teneur moyenne en chlorophylle représentait 91,1 % de la variance de la production primaire de la zone euphotique (ΣA) dans le lac Humboldt (solides dissous totaux (SDT) = $3,3 \text{ g}\cdot\text{L}^{-1}$, $Z_{\text{max}} = 6 \text{ m}$), tandis que la concentration de PT et de phosphore dissous total et la température de l'eau représentaient 82,7 % de la variance de la ΣA dans le lac Redberry (SDT = $20,9 \text{ g}\cdot\text{L}^{-1}$, $Z_{\text{max}} = 17 \text{ m}$). L'importance relative de ces variables pour ΣA s'expliquait par des différences biologiques, chimiques et physiques entre ces lacs. La lumière pénétrait en général jusqu'au fond du lac Redberry en raison de la concentration moyenne de la chlorophylle dans la zone euphotique (Z_{eu}) de $1,7 \text{ mg}\cdot\text{m}^{-3}$, tandis que dans le lac Humboldt, la valeur moyenne de Z_{eu} était de 3,4 m avec une concentration de chlorophylle moyenne de $62,6 \text{ mg}\cdot\text{m}^{-3}$. La chlorophylle était le facteur dominant corrélé à la pénétration de la lumière dans le lac Humboldt ($r^2 = 0,65$), mais pas dans le lac Redberry. La capacité photosynthétique n'était corrélée ($r^2 = 0,72$) à la température de l'eau que dans le lac Redberry. La ΣA moyenne était respectivement de 57,1 et de 230,2 $\text{mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ pour les lacs Redberry et Humboldt.

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Athalassic saline lakes are distributed around the world wherever evaporation exceeds precipitation and endorheic drainage exists (Hammer 1978). According to Hammer, saline waters occur on every continent but are most prominent in Africa, Asia, Australia, and North America. Hammer (1986) has noted that the volume of the world's saline lakes is approximately equal to that of freshwaters.

Rates of phytoplankton photosynthesis, or primary productivity, can vary widely in saline lakes, with hypersaline lakes tending to be less productive (Hammer 1986). Jellison and Melack (1988) noted that while there are numerous descriptions of daily and seasonal fluctuations in primary production in saline waters, few studies exist on year-to-year changes or contain statistical analysis of the relationships between primary production and environmental factors known to regulate levels of production. A similar point was made by Hammer (1981).

Studies correlating environmental factors with changes in primary production are particularly required for saline waters in which sulphate is the dominant anion. Primary production in freshwater systems with low sulphate concentrations tends

to be phosphorus limited whereas production in many saline systems with high sulphate concentrations is often phosphorus sufficient and nitrogen deficient (Caraco et al. 1989). Caraco et al. concluded that this was due to increased phosphorus release from sediments at high sulphate concentrations. In prairie saline lakes, phytoplankton chlorophyll *a* and primary production were found by Campbell and Prepas (1986) to be much lower than predicted from ambient phosphorus levels and empirical models developed for freshwater lakes. Mg^{2+} and SO_4^{2-} are relatively more important in the saline lakes of western Canada than elsewhere in the world (Campbell and Prepas 1986). The relative importance of Mg^{2+} and SO_4^{2-} appears to be a major factor accounting for low productivity in saline lakes of western Canada and high productivity in saline lakes elsewhere (Campbell and Prepas 1986).

In the present study, primary production was measured for two years in two south-central Saskatchewan lakes with marked differences in their biological, chemical, and physical characteristics. Redberry Lake ($52^\circ 43' \text{N}$, $107^\circ 09' \text{W}$) is saline (total dissolved solids (TDS) = $20.9 \text{ g}\cdot\text{L}^{-1}$), 17 m deep, and oli-

gotrophic (maximum euphotic zone (Z_{eu}) $Chl = 5.5 \text{ mg} \cdot \text{m}^{-3}$, whereas Humboldt Lake ($52^{\circ}09'N$, $105^{\circ}06'W$) is hypertrophic (maximum Z_{eu} $Chl = 839 \text{ mg} \cdot \text{m}^{-3}$), 6 m deep, and slightly saline ($TDS = 3.3 \text{ g} \cdot \text{L}^{-1}$). The water columns of both lakes remain aerobic throughout the year. Sulphate is the dominant anion in both Humboldt (87.3%) and Redberry (93.1%) lakes with concentrations of 27 274 and 130 208 μM , respectively (Hammer 1978). On the basis of Caraco et al.'s (1989) analysis, primary production in Humboldt and Redberry lakes should be nitrogen limited. The lakes are described in more detail in Rawson and Moore (1944), Hammer and Haynes (1978), and Timms et al. (1986).

Concurrent with in situ estimates of primary production, we measured a wide range of environmental variables known to influence primary production in fresh and marine waters (Harris 1978; Reynolds 1984) including algal biomass and species composition, underwater light climate, nutrient concentrations, and water temperature. We used statistical analysis to identify whether light, nutrients, and temperature were correlated with intrinsic, as well as areal and volumetric, production parameters.

Materials and Methods

Water samples were collected from a central, deepwater station in Redberry (16 m) and Humboldt (6 m) lakes with an opaque 8-L Niskin water sampler (internal diameter = 7 cm; length 71.5 cm). In Redberry Lake, the sampler was held vertically and samples were collected from the surface to the bottom at 2-m intervals. In Humboldt Lake the sampler was operated horizontally, because of frequent sharp phytoplankton gradients, and water was collected from the surface, at 0.5 m, and then from 1-m intervals to the bottom. The temporal sampling intervals were biweekly in Redberry Lake from May to October 1989 and biweekly in Humboldt Lake from May to June 1989, weekly from July to September and again biweekly to mid-October. Both lakes were sampled during ice-cover in January, February and March. Water samples were collected from surface (just beneath the ice) and 8 and 16 m in Redberry Lake and the surface and 6 m in Humboldt Lake. Unstable ice conditions prevented sampling in the intervening months. Sampling was done on a monthly basis at both lakes in summer 1990.

Physical Measurements

Total incident radiation was measured with Moll-Gorczynski-type solarimeters (Kipp and Zonen, Delft, Holland). These data were corrected assuming an average 5% surface reflectance and to photosynthetically available radiation (PAR, 400–700 nm) using a factor of 0.46 (Talling 1965) and were used to calculate intrinsic photosynthetic parameters (see below). The solarimeter at Humboldt Lake was mounted in an open shore-side area, while that at Redberry Lake was placed on an island.

The attenuation of PAR in the water was measured with a pair of cosine-corrected quantum irradiance probes (LI-192SB, Lambda Instruments Co.) connected to a quantum meter (LI-185B), one to measure downwelling irradiance and the other to measure upwelling irradiance. In addition, a deck quantum sensor (LI-190SB) was used to measure variations in surface irradiance due to changing cloud conditions while underwater measurements were being made. Underwater readings were from just beneath the surface and thereafter at 1-m intervals to the bottom in Redberry Lake. In Humboldt Lake, measure-

ments were made just beneath the surface and at 25- to 50-cm intervals to the depth of 1% of the subsurface PAR value (Z_{eu}). The attenuation coefficient, K_d (in units per metre) and Z_{eu} were calculated by linear regression (Kirk 1977). Three estimates of scattering properties for each lake were calculated according to Kirk (1977): reflectance (R_s), which is the ratio of upwelling to downwelling PAR at each depth; asymptotic reflectance (R_a), which is the asymptote value in the depth profile of R_s values; and the backscattering coefficient (b'_b), which is calculated as $b'_b = R_a \cdot 2K_d$.

Water transparency was measured with a standard 20-cm-diameter black and white Secchi disc, while temperature was measured at about midday with a Cole-Parmer 8502-20 thermometer at the depth intervals given above.

Chemical Measurements

pH was measured with a Cole-Parmer portable meter and total alkalinity was determined by titration with 0.1 M HCl to an end-point of pH 4.5.

Water samples taken from 0 and 6 m in Humboldt Lake and from 0, 10, and 16 m in Redberry Lake were analyzed for particulate and dissolved nitrogen and phosphorus, particulate organic carbon (POC), and silica. Additional samples for $\text{NO}_2 + \text{NO}_3\text{-N}$ and soluble reactive phosphorus (SRP) were collected from 4 m in Humboldt Lake and from 4 and 14 m in Redberry Lake. Water was placed in an acid-washed beaker and aliquots were treated in the following manner prior to analysis: samples for total phosphorus (TP) and $\text{NH}_3\text{-N}$ were run through 153- μm pore-size Nitex screens to remove macrozooplankton. Water for total dissolved phosphorus (TDP), SRP, $\text{NO}_2 + \text{NO}_3\text{-N}$, and silica were filtered through precombusted 47-mm GF/C filters. Filtrates were placed in acid-washed bottles and stored on ice in the dark for transport to the laboratory. Material for particulate carbon and particulate nitrogen was collected on 25-mm combusted GF/C filters and then washed with 0.05 M H_2SO_4 . These filters were placed in petri dishes and transported to the laboratory with the other samples. At the laboratory, inorganic nutrients were analyzed using Technicon AutoAnalyzers, while the organic nutrients were analyzed with a CHN analyzer following methods in Environment Canada (1992).

Aliquots of 100–1000 mL from Humboldt Lake samples and 2 L from Redberry Lake samples were drawn through GF/C filters. Filters were placed in petri dishes, wrapped in aluminum foil, and placed on ice in the dark for transport to the laboratory. Chlorophyll *a* was extracted in boiling (78°C) 90% ethanol and determined either spectrophotometrically (Humboldt Lake) or fluorometrically (Redberry Lake) after correction for phaeopigments (Nusch 1980). Total chlorophyll concentrations (ΣB , milligrams per square metre) for the water column and for the euphotic zone were determined by planimetric integration of the appropriate depth profiles. The mean euphotic zone chlorophyll concentration, $\bar{B}$ (milligrams per cubic metre), was calculated as $\Sigma B/Z_{eu}$.

Phytoplankton Measurements

Phytoplankton cell numbers and volumes were determined for integrated depth zones: 0–8 and 10–16 m in Redberry Lake and 0–3 and 4–6 m in Humboldt Lake. Integrated samples were prepared by collecting and combining 100-mL aliquots from each of the depths in the integrated zone. After mixing, a 100-mL subsample was placed in an opaque bottle and preserved with Lugol's solution. For counting, subsamples of

1–10 mL were placed in glass sedimentation chambers and cells were enumerated with an inverted microscope. A combined minimum of 500 cells, colonies, and filaments were counted per sample. Details of the counting procedures and calculation of biomass values from the linear dimensions of cells are given in Earl et al. (1987).

Primary productivity of the euphotic zone was measured using the ^{14}C light and dark bottle technique. Two light bottles and one dark bottle for each depth were filled under shaded conditions and then placed in a light-tight box until all samples were collected. The bottles were then injected with $\text{NaH}^{14}\text{CO}_3$ (Humboldt Lake, 74 kBq per 125-mL bottle; Redberry Lake, 296 kBq per bottle), shaken, attached to clips on clear Plexiglas discs, and incubated over midday at the collection depths. The discs held the bottles parallel to the surface. The incubation harness was suspended from a bar attached to two floats which were far enough apart to prevent shading of the bottles. Incubation times were about 2 and 4 h for Humboldt and Redberry lakes, respectively.

Following injection of the light and dark bottles, aliquots of the ^{14}C solution were injected into a CO_2 trapping agent to verify the concentration of ^{14}C added to the bottles.

After the incubation period, the bottles were retrieved, placed in a light-tight box, and filtered on shore. The amount of water filtered through 0.45- μm pore-size filters varied depending on the concentration of phytoplankton present: 5–100 mL for Humboldt Lake and 100 mL for Redberry Lake. Filters were then placed in glass scintillation vials containing 0.5 mL of 0.5 M HCl (Lean and Burnison 1979) to remove unincorporated ^{14}C . After being left overnight in a fumehood, 10 mL of Filter Count (Packard Instruments) was added to dissolve the filters. The ^{14}C in the vials was counted in a liquid scintillation spectrometer (Packard Instruments, model 1900CA) and counts were corrected for quench and machine efficiency using an external standard.

During the summer cyanobacterial blooms on Humboldt Lake, the filters were treated in the following manner. After being acidified and dried in the fumehood, the cyanobacteria on the filters were dissolved at 50°C overnight in 1 mL of Soluene 350 (United Technologies Packard). Filter Count (15 mL) was then added and the vials were placed in a refrigerator overnight to permit decay of any chemiluminescence. The vials were counted as above, but the counting program was altered to detect and correct for luminescence and colour quenching.

Primary production was calculated from ^{14}C incorporation rates and ^{12}C concentrations available to phytoplankton, estimated from temperature, pH, and alkalinity data and the conversion table of Saunders et al. (1962). Hourly integral production (ΣA , milligrams carbon per square metre per hour) was obtained planimetrically from plots of depth profiles of volumetric rates for May–October. Daily production rates ($\Sigma \Sigma A$) were calculated using the equation given by Talling (1965) and these were planimetrically integrated to give annual primary production rates.

Photosynthesis per unit chlorophyll (A/B , milligrams carbon per milligram chlorophyll per hour) versus irradiance (see above) curves were plotted for each measurement date. Alpha (α , the slope of the initial linear region of the rate–irradiance curve (milligrams carbon per milligram chlorophyll per hour/microeinstiens per square metre per second)) was calculated by linear regression and I_k , the irradiance (microeinstiens per square metre per second) indicating the onset of light saturation of photosynthesis and equivalent to the light intensity at which

an extrapolation of α would reach the measured light-saturated rate (A_{max}/B), was calculated as $A_{\text{max}}/B/\alpha$.

Relative measures of photosynthetic potential beneath the ice were made using modifications of the above light and dark bottle technique. Holes were drilled in the ice and water samples were collected from just below the ice. After filling the bottles and injecting them with ^{14}C , three sets of two light bottles each were wrapped in shade cloth to give relative light values of 27, 7, and 2% of full light. PAR reaching the water of Redberry Lake in winter was 2.5–5.5% of the above-ice value due to snow and ice cover in excess of 1 m, while in Humboldt Lake, ice and snow cover (0.5–1 m) reduced PAR to 2.3–15.3% (virtually no snow) of above-ice PAR values. An additional two bottles had no shade cloth and two others were opaque. All the bottles were incubated for 3–4 h in an open box containing lake water which was frequently changed to prevent freezing. The contents of the bottles were filtered in the field and the filters were processed as described above.

Results

Water Temperature

Humboldt Lake did not have typical dimictic stratification patterns because its maximum depth was only 6 m. Wind activity usually kept this lake mixed, but occasionally, diurnal mixed layers developed on hot summer days (see Fig. 6). At the surface, the minimum water temperature was -0.5°C in February, while the highest temperature (25.1°C) was recorded in late July/early August. The bottom waters had a similar temperature range of 0.5 – 22.2°C .

Redberry Lake developed a pronounced thermocline (see Fig. 4) and was thermally stratified from May to September–October. Surface water temperatures ranged from -1.0 (January) to 21.8°C (July). At 16 m the water temperature rarely exceeded 4°C except during fall overturn when a temperature as high as 7.6°C was measured in October.

Underwater Light Penetration

Redberry Lake water was significantly more transparent than that of Humboldt Lake. The attenuation coefficient of PAR (K_d) in Redberry Lake ranged between $0.230\cdot\text{m}^{-1}$ in July to $0.398\cdot\text{m}^{-1}$ in June, giving a mean Z_{eu} of 15.2 m (Table 1). On 13 of 22 measurement dates, Z_{eu} was calculated to equal or exceed the depth of the lake so that as much as 7.5% of surface light reached the bottom sediments. R_a varied between 0.033 in July to 0.117 in June but was usually <0.050 , while b'_b was 0.016–0.056 for the same months.

In contrast, K_d for Humboldt Lake was lowest in June and greatest in August with values of 0.618 and $3.304\cdot\text{m}^{-1}$, respectively. The euphotic zone extended to the bottom on four dates (late May and June), while the mean Z_{eu} was 3.4 m (Table 1). On 1 August 1989, a major scum of cyanobacteria was encountered with a surface concentration of $839.3\text{ mg Chl}\cdot\text{m}^{-3}$. This scum moved off the sampling station shortly after all water samples were collected. This resulted in a major change in the underwater light field with the Secchi disc value increasing from 0.2 m in the scum to 1.6 m after it moved away. This biased not only the light data but also the chlorophyll and primary production data (see below); consequently, these data were not included in the following analyses. R_a varied between 0.032 in May–June to 0.068 in October, while b'_b was 0.040 in June and 0.425 in July.

TABLE 1. Underwater light characteristics (symbols defined in the text) and the mean chlorophyll *a* concentration of the euphotic zone for Humboldt and Redberry lakes during the ice-free periods in 1989 and 1990. Data are means, standard deviations and coefficients of variation.

Lake	K_d (m^{-1})	Z_{eu} (m)	R_a	b'_b	Chl <i>a</i> ($mg \cdot m^{-3}$)
Humboldt	1.619	3.4	0.045	0.152	62.6
	0.854	1.5	0.015	0.099	63.7
	53%	45%	33%	65%	102%
Redberry	0.290	15.2	0.050	0.030	1.7
	0.043	1.6	0.019	0.013	1.3
	15%	11%	39%	44%	77%

Water Chemistry

Marked differences in the nutrient levels (Table 2) of the two lakes accounted for the differences in the underwater light regimes. There were no distinct seasonal cycles in Redberry Lake of TDP, SRP, and silica concentrations, but silica tended to be lowest in July–August each year. The concentration of $NO_2 + NO_3-N$ was usually $<2 \mu g \cdot L^{-1}$ but increased to 12–15 $\mu g \cdot L^{-1}$ beneath the ice. NH_3-N was mostly $<30 \mu g \cdot L^{-1}$ and was also greatest beneath the ice with concentrations of 47–58 $\mu g \cdot L^{-1}$. In addition, even though there was a marked thermocline throughout summer (see Fig. 4), there was no significant variation in nutrient concentrations with depth.

Part-N and particulate organic carbon (POC) in Redberry Lake (Table 2) were at their lowest concentrations during winter, and concentrations of both parameters were frequently greatest at 16 m. These seasonal variations were also exhibited by the chlorophyll *a* data (Fig. 1).

Unlike the chemical data from Redberry Lake, most of the nutrient parameters (Table 2) in Humboldt Lake had distinct seasonal cycles. $NO_2 + NO_3-N$ and NH_3-N were low during May, increased in June, and then continued to decline into August. In September–October, these nutrients began to increase, reaching maximum values under the ice. TP did not have a distinct seasonality, but SRP was lowest in May after which concentrations climbed into July, remaining high (250–300 $\mu g \cdot L^{-1}$) until late August when they began to decline, reaching values of about 130 $\mu g \cdot L^{-1}$ in October. Under the ice, SRP increased to about 240 $\mu g \cdot L^{-1}$. The silica concentration was markedly different in the two years: in 1989, it reached 7710 $\mu g \cdot L^{-1}$ by August and dropped to 50 $\mu g \cdot L^{-1}$ in September and October. In 1990, the maximum silica concentration was 2020 $\mu g \cdot L^{-1}$ in July but for the rest of the year was frequently $<500 \mu g \cdot L^{-1}$.

In accordance with the higher nitrogen and phosphorus concentrations, Part-N and POC were markedly greater in Humboldt Lake than in Redberry Lake (Table 2). Both Part-N and POC were high in early May, low in late May through to July, and remained high from mid-July until February when Part-N dropped to about 35 $\mu g \cdot L^{-1}$ and POC to $<300 \mu g \cdot L^{-1}$. These

seasonal changes were mirrored by the chlorophyll *a* concentrations (Fig. 1).

With the exception of SRP ($r = 0.22$, $n = 102$, $p < 0.05$), there was no significant correlation between nitrogen, phosphorus, or silica concentration and chlorophyll concentration for Redberry Lake. In Humboldt Lake, changes in chlorophyll concentration were positively correlated with silica concentration ($r = 0.53$, $n = 24$, $p < 0.01$) and inversely with $NO_2 + NO_3-N$ (Fig. 2) and NH_3-N ($r = -0.64$, $n = 24$, $p < 0.001$).

Changes in phytoplankton standing crop (chlorophyll *a*) had a strong influence on the underwater light climate in Humboldt Lake (Fig. 3) but not in Redberry Lake ($r = 0.10$, $n = 22$, $p > 0.5$). The slope of the regression between chlorophyll concentration and K_d is the chlorophyll specific attenuation coefficient ($K_s = 0.011 m^2 \cdot mg^{-1}$), while the intercept is K_w (0.94 $m^2 \cdot mg^{-1}$) and represents the proportion of light extinction due to factors other than chlorophyll. Both R_a ($r = 0.63$, $n = 24$, $p = 0.001$) and b'_b ($r = 0.85$, $n = 24$, $p < 0.001$) were influenced by changes in chlorophyll concentration in Humboldt Lake but not in Redberry Lake (for R_a , $p > 0.05$; for b'_b , $p = 0.10$) (Table 1).

Phytoplankton

The species composition of the phytoplankton populations in the two lakes were different as anticipated from the differences in nutrient chemistry. In addition, there was a major change in the dominant summer cyanobacterial species in Humboldt Lake between 1989 and 1990.

In Redberry Lake in 1989, chrysophytes (*Chrysoidalis peritaphrena*, *Cercobodo varians*) were dominant after ice-out in May, in the hypolimnion throughout the year, and under the ice. In the surface populations, they formed up to 69% of the biomass and as much as 84% of the hypolimnetic population. Diatoms (*Stephanodiscus niagarae* Ehr., *Chaetoceros elmorei* Boyer, unidentified centric diatom(s)) composed $>88\%$ of the phytoplankton biomass in June–July and late August/September and were also dominant under the ice in January. Chlorophytes, mainly *Sphaerocystis Schroteri* Chodat and *Oocystis*

TABLE 2. Phosphorus and nitrogen concentrations ($\mu g \cdot L^{-1}$) in Humboldt and Redberry lakes from May 1989 to October 1990. Data are means and ranges for all depths and dates; no mean value is given for $NO_2 + NO_3-N$ in Redberry Lake, as on most occasions it was $<2 \mu g \cdot L^{-1}$, the detection limit of the method. Abbreviations of the chemical names are explained in the text.

Lake	TDP	SRP	$NO_2 + NO_3-N$	NH_3-N	Silica	TP	Part-N	POC
Redberry	40	21	—	26	310	47	34	367
	26–75	11–68	$<2-15$	14–67	20–310	27–101	10–154	100–996
Humboldt	259	206	32	150	3630	308	431	3083
	92–380	24–327	$<2-139$	38–568	20–7710	147–443	10–1440	241–7510

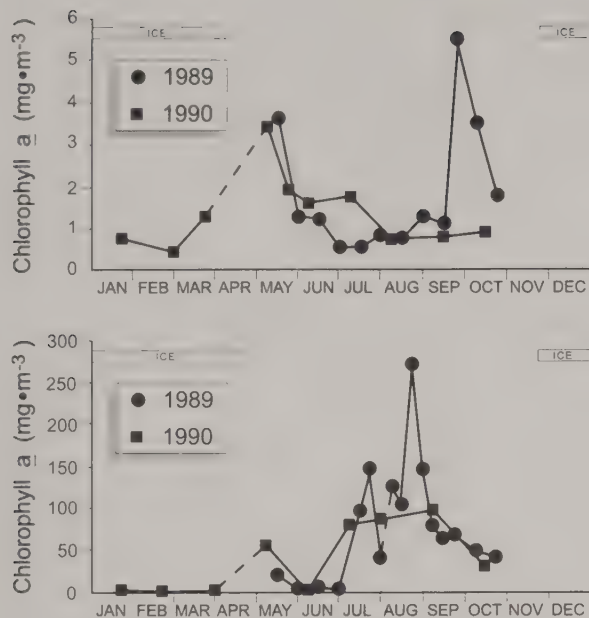


FIG. 1. Seasonal changes in the mean euphotic zone chlorophyll *a* concentration in Redberry (upper panel) and Humboldt (lower panel) lakes in 1989 and 1990. Broken lines indicate missing data points. Note differences in scale.

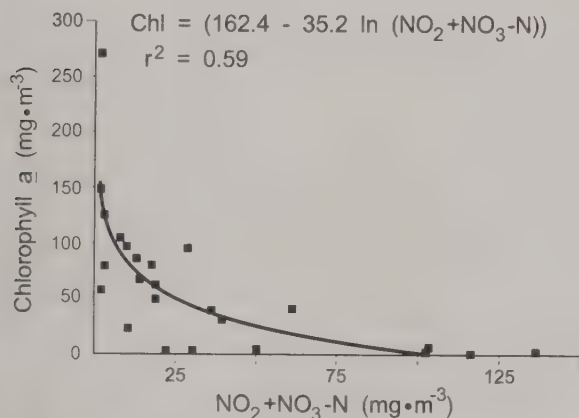


FIG. 2. Relationship between the mean euphotic zone chlorophyll *a* concentration and the concentration of $\text{NO}_2 + \text{NO}_3\text{-N}$ in Humboldt Lake in 1989 and 1990.

borgei Snow, formed up to 87% of the biomass in July and again in October. On August 10, *Aphanizomenon flos-aquae* Ralfs had a biomass of $214 \text{ mg} \cdot \text{m}^{-3}$ which was 45% of the total biomass. Cryptophytes were never dominant and euglenophytes formed only a minor part of the population. In 1990 chlorophytes, mainly *Coccomyxa minor* and other unidentified *Coccomyxa* species, were dominant for most of the year. On 4 June the dominant phytoplankton species was *Chaetoceros elmorei* Boyer, forming 64% of the biomass. Chrysophytes dominated the hypolimnetic population in May–June, while diatoms dominated in July–August.

In Humboldt Lake, diatoms consisting of an unidentified centric species and *Stephanodiscus niagarae* formed 42.8–

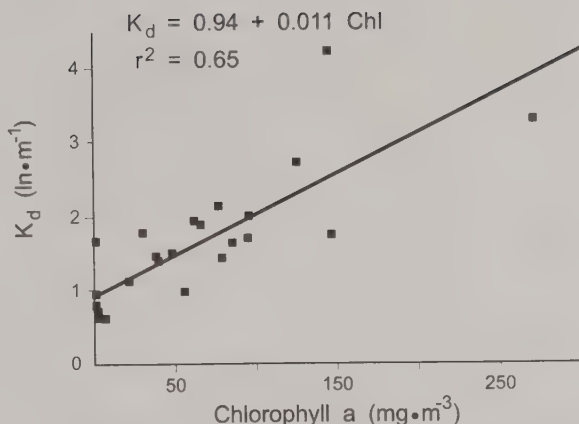


FIG. 3. Relationship between the mean euphotic zone chlorophyll *a* concentration and the attenuation coefficient (K_d) of PAR in Humboldt Lake in 1989 and 1990.

66.8% of phytoplankton biomass from May to mid-June 1989. From June to 8 August, *Aphanizomenon flos-aquae* formed 61–99.8% of the biomass. For the rest of the ice-free period, *Stephanodiscus* was the dominant species with *Aphanizomenon* as the co-dominant. Under the ice the cryptophyte *Rhodomonas lens* Skuja and the chrysophyte *Ochromonas polychrysis* Skuja tended to be the dominant contributors to the phytoplankton biomass until March 1990 when the cyanobacterium *Gleothoece rupestris* (Lyngb.) Born. bloomed. An unidentified centric diatom was dominant in May 1990 followed by *Gleothoece rupestris* which was dominant for the remainder of the year.

Primary Productivity

The maximum volumetric rate of primary production (A_{max}) in Redberry Lake varied from $3.2 \text{ mg C} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ in June 1990 to $31.6 \text{ mg C} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ in August 1989. *Aphanizomenon flos-aquae* was primarily responsible for this production peak. Under the ice, a maximum potential production rate of only $0.5 \text{ mg C} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ was measured. The depth profiles of production (Fig. 4) were characteristic of oligotrophic lakes, with one or more production peaks distributed over a deep euphotic zone. Even though the chlorophyll maximum was frequently located in the hypolimnion, this region did not sustain high rates of production due to low water temperatures and light levels (see below).

Maximum Z_{eu} primary productivity (ΣA , milligrams carbon per square metre per hour) in Redberry Lake occurred in August 1989, while in 1990 it occurred in September with the fall bloom (Fig. 5). Daily productivity rates were between 246.2 and $975.5 \text{ mg C} \cdot \text{m}^{-2}$, giving annual productivity rates of $93.7 \text{ g C} \cdot \text{m}^{-2}$ for 1989 and $68.1 \text{ g C} \cdot \text{m}^{-2}$ for 1990.

REDBERRY LAKE

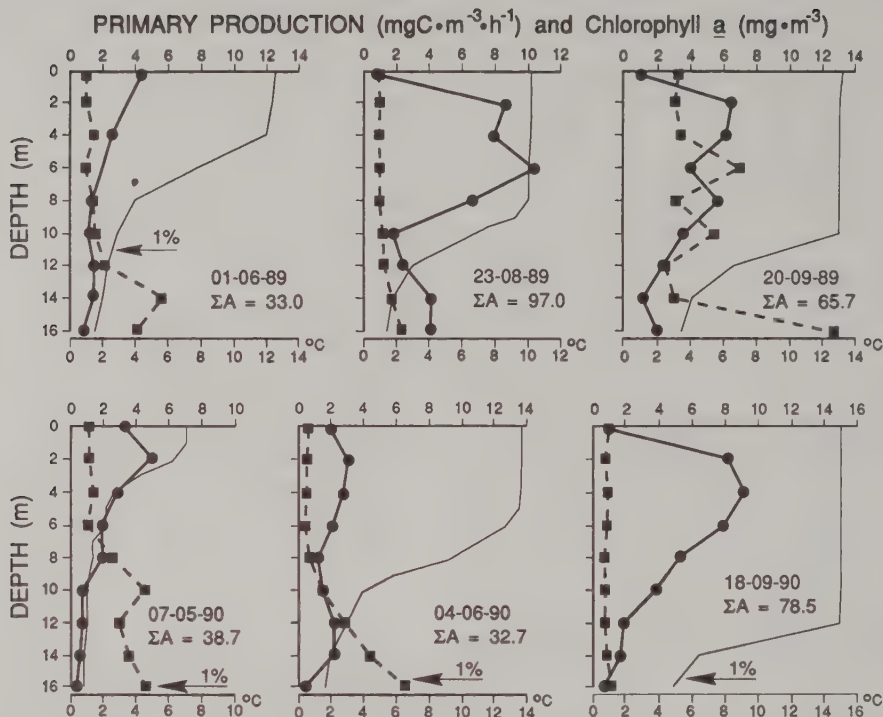


FIG. 4. Representative depth profiles of primary production (circles), chlorophyll *a* concentration (squares), and water temperature (thin line) in Redberry Lake. ΣA ($\text{mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) is the integrated rate of production for the euphotic zone, the depth where 1% of surface PAR remained (arrows); the absence of an arrow indicates that light penetrated to the bottom sediments.

Primary productivity depth profiles in Humboldt Lake were characteristic of eutrophic lakes, with A_{max} ($1.7\text{--}531.4 \text{ mg C} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$) usually being found in the top metre of the water column (Fig. 6). Under the ice, potential volumetric rates varied from $0.9 \text{ mg C} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ in January to $5.4 \text{ mg C} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ in March. By March the chlorophyll had increased to $2.6 \text{ mg} \cdot \text{m}^{-3}$ from $\leq 0.5 \text{ mg} \cdot \text{m}^{-3}$ (Fig. 1) under a snowless ice cover of about 1 m.

Although the euphotic zone in Humboldt Lake was markedly shallower than that of Redberry Lake, high levels of primary productivity were maintained because of the large phytoplankton standing crops. ΣA was lowest ($4.0 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) during the clear-water phase in June 1990 following the spring bloom and highest ($503.4 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) during the *Aphanizomenon* bloom in August 1989 (Fig. 5). On 1 August, an *Aphanizomenon* scum produced an A_{max} rate of $1864.2 \text{ mg C} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ and a ΣA rate of $2390.7 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$. However, due to the artificial underwater light regime produced by the removal of this scum (see Materials and Methods), these data have not been included in Fig. 5 or the following analysis. The calculated daily rates of primary productivity varied from $0.05 \text{ g C} \cdot \text{m}^{-2}$ in June 1990 to $4.38 \text{ g C} \cdot \text{m}^{-2}$ in August 1989, giving annual productivities of $294.8 \text{ g C} \cdot \text{m}^{-2}$ for 1989 and $562.1 \text{ g C} \cdot \text{m}^{-2}$ for 1990.

The relationship between A_{max} and ΣA for Redberry ($r = 0.96, p < 0.001$) and Humboldt ($r = 0.93, p < 0.001$) lakes was linear. ΣA was not correlated with K_d ($r = 0.45, p > 0.05$) in Redberry Lake but was correlated with K_d in

Humboldt Lake ($r = 0.67, p = 0.001$). Consequently, chlorophyll concentration was not a good predictor of primary production in Redberry Lake ($r = 0.15, p > 0.5$) but was a fairly strong predictor ($r = 0.79, p < 0.001$) for Humboldt Lake. Water temperature was correlated with ΣA in both Redberry ($r = 0.67, p = 0.002$) and Humboldt ($r = 0.57, p = 0.005$) lakes. Solar radiation was not correlated ($p > 0.2$) with ΣA for either lake.

Intrinsic Rates of Primary Production

Photosynthetic capacity, A_{max}/B , ranged from 1.9 to $19.7 \text{ mg C} \cdot (\text{mg Chl})^{-1} \cdot \text{h}^{-1}$ in Redberry Lake and was strongly correlated with water temperature (Fig. 7). The Q_{10} between 10 and 20°C for this relationship was 3.3 . A_{max}/B was not correlated ($p > 0.1$) with any of the chemical parameters measured. In contrast, A_{max}/B for Humboldt Lake phytoplankton varied from 1.3 to $6.2 \text{ mg C} \cdot (\text{mg Chl})^{-1} \cdot \text{h}^{-1}$, was not correlated ($r = 0.37, p > 0.1$) with water temperature, was correlated with silica concentration ($r = 0.54, p = 0.02$), but was not correlated with other chemical parameters.

The mean value for the saturation parameter, I_k , for Redberry Lake phytoplankton was $192.9 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (range $51.4\text{--}380.4$) compared with a mean value of $358.0 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (range $114\text{--}780.1$) for Humboldt Lake phytoplankton (Table 3). The highest I_k values in Humboldt Lake always occurred with the summer cyanobacterial blooms. Both solar radiation levels and water temperature were correlated with I_k values in both lakes (Humboldt Lake: solar radiation, $r = 0.75$,

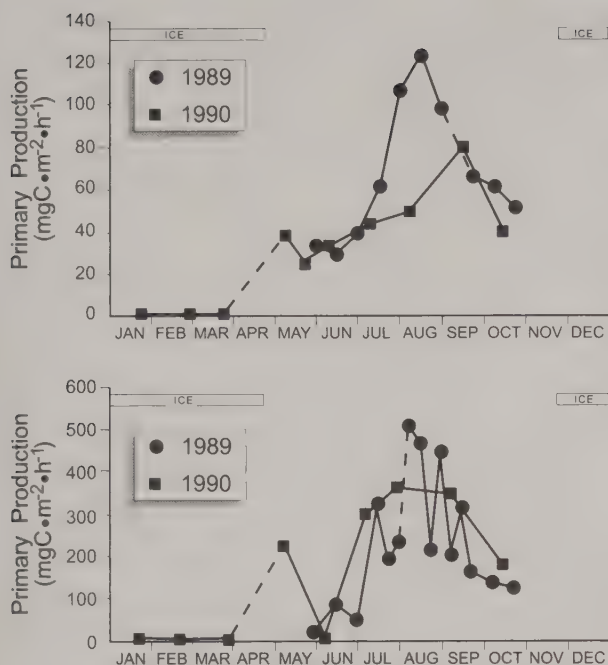


Fig. 5. Seasonal changes in the rate of euphotic zone primary production in Redberry (upper panel) and Humboldt (lower panel) lakes in 1989 and 1990. Broken lines indicate missing data points.

$p < 0.001$; temperature, $r = 0.59$, $p < 0.02$; Redberry Lake: solar radiation, $r = 0.70$, $p < 0.005$; temperature, $r = 0.64$, $p < 0.02$).

Photosynthetic efficiency (α) for Redberry Lake phytoplankton had a mean value of $0.046 \text{ mg C} \cdot (\text{mg Chl})^{-1} \cdot \text{h}^{-1} \cdot (\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1})^{-1}$ (range 0.015–0.121) (Table 3). Alpha had a fairly strong correlation with water temperature ($r = 0.76$, $p < 0.001$) but was not correlated ($p > 0.1$) with solar radiation.

The phytoplankton of Humboldt Lake exhibited a lower α than the plankton of Redberry Lake with a mean value of $0.013 \text{ mg C} \cdot (\text{mg Chl})^{-1} \cdot \text{h}^{-1} \cdot (\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1})^{-1}$ (range 0.005–0.047) (Table 3). The lowest values usually occurred in summer with the cyanobacterial bloom and the higher values occurred in October. There was no significant correlation between α and water temperature, but there was an inverse correlation with solar radiation ($r = -0.49$, $p = 0.05$).

Primary Production Model

A stepwise multiple regression analysis using all environmental and nutrient covariables showed that chlorophyll concentration, K_d , TP, and $\text{NH}_3\text{-N}$ accounted for 98.5% of the variance in ΣA for Humboldt Lake. For Redberry Lake, TP, TDP, and water temperature were the dominant factors accounting for 82.7% of ΣA variance (Table 4).

Discussion

With mean primary production rates of 462 and 1994 $\text{mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ (Table 3), Redberry and Humboldt lakes, respectively, would be classified as oligomesotrophic and hypertrophic according to Wetzel (1983). On the basis of its annual production, Humboldt Lake is one of the most productive in

the world, the result of high nutrient loads from treated sewage, cattle feedlot drainage, and agricultural runoff (Hammer 1986).

Haynes and Hammer (1978) have reported measurements of primary production in Redberry and Humboldt lakes for the ice-free periods in 1972 and 1973. They recorded daily production estimates (three measurements) for Redberry Lake of $124\text{--}628 \text{ mg C} \cdot \text{m}^{-2}$. We obtained higher rates of $246\text{--}976 \text{ mg C} \cdot \text{m}^{-2}$. For Humboldt Lake, Haynes and Hammer obtained rates of $263\text{--}7968 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ (nine measurements) which tend to be higher than our range of $50\text{--}4380 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$. Haynes and Hammer's highest rate was obtained from a large population of *Aphanizomenon*. We measured a rate of $18\,930 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ in an *Aphanizomenon* scum but have not included it in our analysis for reasons given above. The annual production rate calculated by Haynes and Hammer for Humboldt Lake was $613 \text{ g C} \cdot \text{m}^{-2}$ and was similar to our value of $562 \text{ g C} \cdot \text{m}^{-2}$ in 1990 but higher than our 1989 value of $295 \text{ g C} \cdot \text{m}^{-2}$. The higher annual rates recorded by Haynes and Hammer and by us in 1990 may have been due to sampling effort. Typical of hypertrophic lakes, buoyant cyanobacterial populations demonstrate large biomass and production fluctuations in relation to changing wind and mixing conditions, as shown by our more intensive data set in 1989 (Fig. 1 and 5; Tables 1 and 3). The lower sampling effort smooths out these variations resulting in greater annual productivities.

Our data and regression model of the factors influencing primary production in Redberry and Humboldt lakes (Table 4) showed major differences between these two systems. Redberry Lake was a clear-water system with low standing crops of phytoplankton which were probably nutrient deficient. Chlorophyll concentration did not have a major regulating role in the control of the underwater light climate, and the attenuation of PAR was not significantly correlated with ΣA . Water temperature was a significant correlate with all the intrinsic photosynthetic parameters (e.g. Fig. 7) similar to the data of Jellison and Melack (1988) for phytoplankton populations in hypersaline Mono Lake, California, which has a similar depth but stratification dynamics very different from Redberry Lake. Phosphorus and water temperature were the key factors regulating water column production (Table 4) in Redberry Lake even though TDP never dropped below $30 \mu\text{g P} \cdot \text{L}^{-1}$ and SRP was seldom $< 15 \mu\text{g P} \cdot \text{L}^{-1}$.

During summer, Humboldt Lake chlorophyll concentration peaked (Fig. 1) with the cyanobacterial blooms and was the period of greatest primary production (Fig. 5). Haynes and Hammer (1978) found that variations in ΣA in Humboldt Lake were largely ($r^2 = 0.88$) accounted for by changes in *Aphanizomenon* volume.

Changes in chlorophyll concentration were a major factor influencing PAR attenuation (Fig. 3) and ΣA (Table 4) in Humboldt Lake. Light attenuation and ΣA were significantly correlated, but the relationship between ΣA and A_{max} was linear. These data indicated that as A_{max} increased with increasing chlorophyll concentration, light attenuation was not limiting the value of ΣA . A fundamental limitation of photosynthetic productivity per unit area (ΣA) is that high activity per unit volume and deep profiles are mutually exclusive (Talling 1982). This limitation, as Talling noted, centres on the ratio between the maximal rate of photosynthesis per unit biomass (A_{max}/B) and the increment of the light attenuation coefficient per unit biomass (K_c), which have opposing effects on the area enclosed by a depth profile, or ΣA . Both K_c and K_w were low for Humboldt Lake compared with other eutrophic lakes and favoured high ΣA values (cf. Megard et al. 1979; Kirk 1986). The phy-

HUMBOLDT LAKE

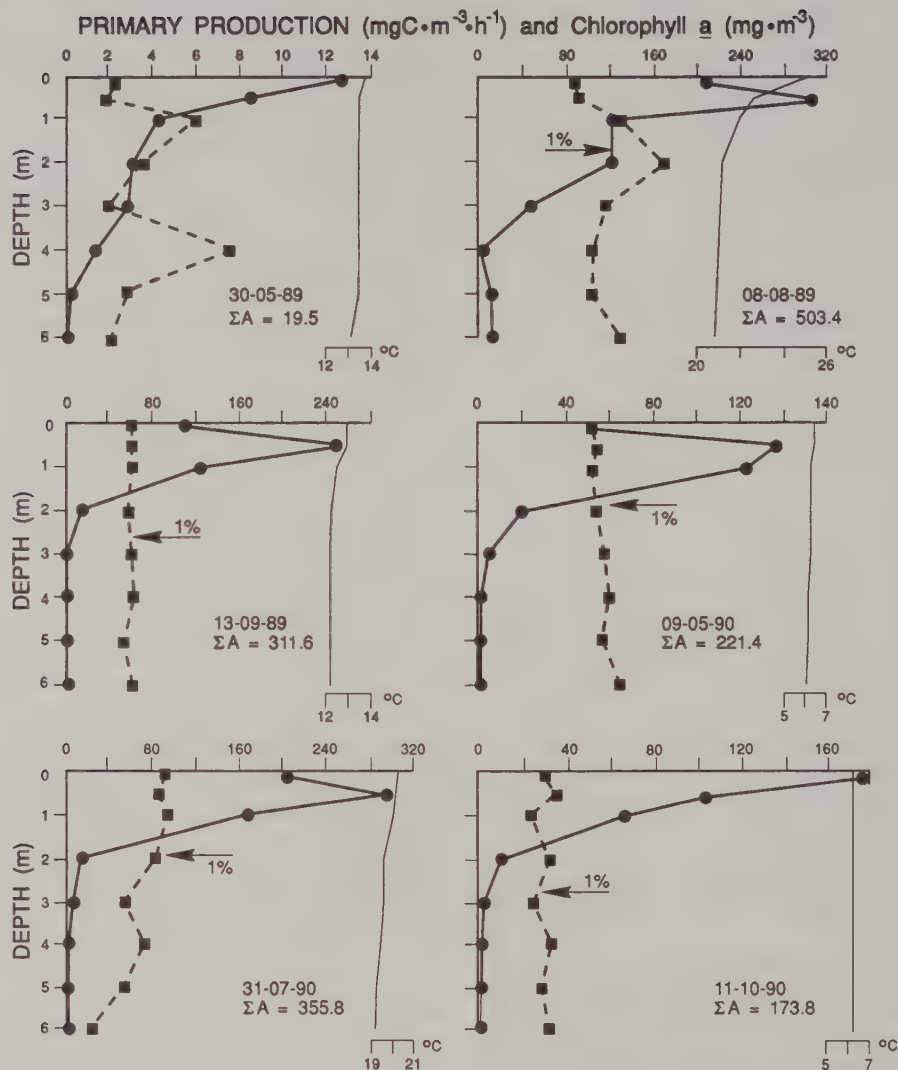


FIG. 6. Representative depth profiles of primary production (circles), chlorophyll *a* concentration (squares), and water temperature (thin line) in Humboldt Lake. ΣA ($\text{mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) is the integrated rate of production for the euphotic zone, the depth where 1% of surface PAR remained (arrows); the absence of arrows indicates that light penetrated to the bottom sediments.

toplankton standing crops in Humboldt Lake were not yet at the level where A_{max} exceeded ΣA due to self-shading as has been found in hypertrophic lakes with much larger standing crops (Robarts and Zohary 1992).

Phytoplankton may respond physiologically to low-frequency variations in light intensity (light-shade adaptation) or light quality (chromatic adaptation) (Falkowski 1984). These responses are manifested in the intrinsic parameters (A_{max}/B , I_k , α) of production (Falkowski et al. 1985). Falkowski noted that changes included variations in amounts and ratios of photosynthetic pigments, changes in photosynthetic responses, gross chemical composition, cell volume, dark respiration rates, and frequently, levels of enzyme activity associated with car-

bon fixation. Depending on species, shade adaptation may result in increased light utilization efficiency, while in others, efficiency may not change or decrease (Falkowski 1984). The ecological significance of light-shade adaptation depends on the interrelationship between vertical mixing rates and adaptation times and, because light quality changes with depth, it is not clear that light-shade and chromatic adaptation can be distinguished or actually differ in phytoplankton (Falkowski 1984).

The range of values for α in the literature is 0.007–0.133 $\text{mg C} \cdot (\text{mg Chl})^{-1} \cdot \text{h}^{-1} \cdot (\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1})^{-1}$ with most values falling between 0.022 and 0.065 (Reynolds 1984). The mean value for Redberry Lake indicated an average light-harvesting ability at

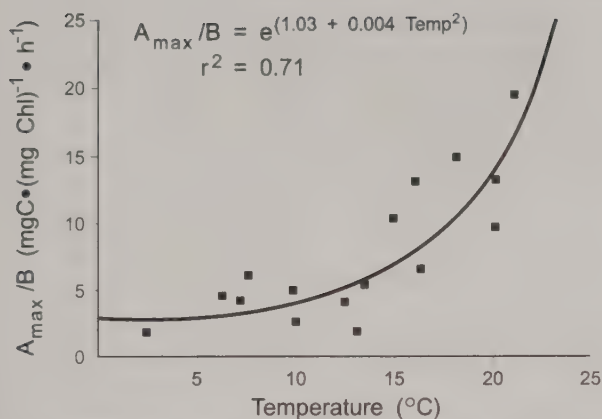


FIG. 7. Relationship between phytoplankton photosynthetic capacity ($A_{\max}/B$) and water temperature in Redberry Lake during the ice-free periods in 1989 and 1990.

low light levels, while the mean value for Humboldt Lake phytoplankton indicated a below-average light-harvesting ability (Table 3).

The literature indicates a range of values for I_k of 20–400 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with a mode occurring between about 60 and 100 (Harris 1978). The mean values from Redberry and Humboldt lakes were 193 and 358 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Table 3), respectively, both indicating light-adapted populations.

Seasonal variations in $A_{\max}/B$ are due mainly to effects of nutrients, temperature, cell size, and light history (Falkowski 1981). In Redberry Lake, $A_{\max}/B$ was correlated with water temperature (Fig. 7), a correlation found for many other phytoplankton populations and primarily related to enzyme kinetics (cf. Harris 1978; Harrison and Platt 1980; Jellison and Melack 1988). For Humboldt Lake, $A_{\max}/B$ was not significantly correlated with temperature but only with silica concentration ($p = 0.02$). A stepwise multiple regression analysis using the environmental and nutrient data with $A_{\max}/B$ for both lakes did not add to the bivariate correlations already found.

Photosynthetic capacity ($A_{\max}/B$) was higher for Redberry Lake than for Humboldt Lake phytoplankton (Table 3). This difference may have been due to a lower chlorophyll content per phytoplankton cell in Redberry Lake, an adaptation strategy for high-light conditions (Falkowski 1981). It is more likely that the low values for Humboldt Lake were an experimental artifact due to the difficulty in obtaining reliable $A_{\max}/B$ rates in compressed phytoplankton canopies using static bottle chains where spatial separation of light-limited and light-inhibited regions was usually small (cf. Robarts and Zohary 1992). The highest $A_{\max}/B$ rates occurred on days when light penetrated the entire water column. The α and I_k data indicated that the phytoplankton from Redberry Lake were physiologically adapted

to lower light conditions than were phytoplankton from Humboldt Lake (Table 3), indicating that the low $A_{\max}/B$ rates from Humboldt Lake were an experimental artifact. This means that ΣA was underestimated by an unknown amount and could account for the lack of correlation between $A_{\max}/B$ and water temperature, as photosynthetic capacity in cyanobacteria is known to be temperature dependent (Robarts and Zohary 1987).

High-light adaptation of the phytoplankton in Humboldt Lake can be ascribed to the dominance of bloom-forming cyanobacteria during summer and the different physical features of the two systems. Bloom-forming cyanobacterial populations accumulate at or near the surface, forming scums during periods of calm when vertical mixing and/or water currents fail to offset cellular buoyancy. Horne et al. (1979) have noted that the buoyancy mechanism of *Aphanizomenon* "flakes" efficiently mitigates most wind-mixing effects. In addition, Humboldt Lake was a shallow ($Z_{\max} = 6$ m, $\bar{Z}$ depth < 4.7 m), polymictic system so that on windy days the phytoplankton were rapidly circulated between surface and bottom. This would have offset the effect of a mean $Z_{\text{eu}}/Z_{\max}$ ratio of 0.53, indicating that phytoplankton spent only about 50% of the time in Z_{eu} .

Redberry Lake, on the other hand, had a $Z_{\max}$ of 17 m with a mean Z_{eu} of 15.2 m (Table 1) and a marked thermocline which began from as high as 4 m below surface (Fig. 4). Essentially the phytoplankton in Redberry Lake were within Z_{eu} at all times. However, the marked thermocline possibly induced physiologically different responses in the phytoplankton contained in the epilimnion and hypolimnion, as indicated by the intrinsic photosynthetic data (Table 3). The epilimnetic population was essentially a warmwater and high-light population: $A_{\max}/B$ always occurred between 2 and 6 m even though the largest biomass frequently occurred near the bottom (Fig. 4). The hypolimnetic population, however, was a relatively low-light and low-temperature population. This resulted in high $A_{\max}/B$ and I_k values from the epilimnetic population and moderate α values, when compared with phytoplankton populations generally, and low chlorophyll-specific production rates from the hypolimnetic population. The construction of single P versus I curves for these two populations is questionable and future studies should separate specific photosynthetic characteristics of epilimnetic and hypolimnetic populations.

The differences in the relative importance of environmental variables regulating primary production that we observed (Table 4) conform to primary production results in fresh and marine waters (cf. Harris 1978; Wetzel 1983; Reynolds 1984; Kirk 1986). Jellison and Melack (1988) found that zooplankton grazing was an important variable to be considered in predicting primary production in Mono Lake. Our simple models predicted less of ΣA variance for Redberry Lake than for Humboldt Lake (Table 4) which may have been due to zooplankton grazing. Grazing could have been a strong factor in Redberry Lake, as the phytoplankton population was dominated by small, edi-

TABLE 3. Volumetric, areal, and intrinsic photosynthetic data calculated (symbols defined in the text) for the phytoplankton of Humboldt and Redberry lakes during the ice-free periods in 1989 and 1990. Data given are means, standard deviations, and coefficients of variation.

Lake	$A_{\max}$	ΣA	$\Sigma \Sigma A$	$A_{\max}/B$	I_k	α
Humboldt	202.0	230.2	1994	3.7	358.0	0.013
	147.7	142.0	1277	1.5	188.9	0.011
	73%	62%	64%	39%	53%	81%
Redberry	7.9	57.1	462	7.9	192.9	0.046
	6.6	28.5	215	5.3	103.1	0.032
	83%	50%	47%	67%	53%	69%

TABLE 4. Multiple regression coefficients for ΣA ($\text{mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) versus environmental covariates in Humboldt and Redberry lakes in 1989 and 1990. Equation constants were 80.43 for Redberry Lake and -87.10 for Humboldt Lake. Chlorophyll = mean concentration ($\text{mg} \cdot \text{m}^{-3}$) for the euphotic zone; nutrient concentrations are surface values.

Lake	Covariate	Coefficient	r^2	p
Humboldt	Chlorophyll	1.90	91.1	0.000
	K_d	62.95	3.3	0.002
	$\text{NH}_3\text{-N}$	-0.38	2.5	0.002
	TP	0.55	1.6	0.002
			98.5	
Redberry	TP	-3.24	38.8	0.000
	TDP	2.43	30.0	0.006
	Temperature	1.73	13.9	0.008
			82.7	

ble species and grazing should be incorporated into our model to increase its predictive capability.

Bierhuizen and Prepas (1985) demonstrated that chlorophyll concentrations are low relative to TP concentrations in western saline lakes. In agreement, our lakes exhibited lower mean summer chlorophyll concentrations (Redberry = $1.7 \text{ mg} \cdot \text{m}^{-3}$; Humboldt = $78.1 \text{ mg} \cdot \text{m}^{-3}$) compared with nonsaline lakes of similar summer TP (Table 2; cf. fig. 1, Bierhuizen and Prepas 1985). On the basis of TP, our data also support Campbell and Prepas's (1986) conclusion that low algal productivity in saline lakes with high sulphate concentrations is influenced by some aspect of salinity which affects nutrient utilization. For managers predicting water quality or fish yields using empirical models developed for freshwaters, our data indicate that the predictions could be serious overestimates. Such models need to be recalibrated for saline lakes with high sulphate concentrations as has been done on a limited scale by Bierhuizen and Prepas (1985).

Acknowledgements

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References

- BIERHUIZEN, J. F. H., AND E. E. PREPAS. 1985. Relationship between nutrients, dominant ions, and phytoplankton standing crop in prairie saline lakes. *Can. J. Fish. Aquat. Sci.* 42: 1588–1594.
- CAMPBELL, C. E., AND E. E. PREPAS. 1986. Evaluation of factors related to the unusually low chlorophyll levels in prairie saline lakes. *Can. J. Fish. Aquat. Sci.* 43: 846–854.
- CARACO, N. F., J. J. COLE, AND G. E. LIKENS. 1989. Evidence of sulphate-controlled phosphorus release from sediments of aquatic systems. *Nature (Lond.)* 341: 316–318.
- EARLE, J. C., H. C. DUTHIE, AND D. A. SCRUTON. 1987. Factors influencing the distribution of phytoplankton in 97 headwater lakes in insular Newfoundland. *Can. J. Fish. Aquat. Sci.* 44: 639–649.
- ENVIRONMENT CANADA. 1992. Analytical methods manual. Inland Waters Directorate, Water Quality Branch, Environment Canada, Ottawa, Ont.
- FALKOWSKI, P. G. 1981. Light-shade adaptation and assimilation numbers. *J. Plankton Res.* 3: 203–216.
- . 1984. Physiological responses of phytoplankton to natural light regimes. *J. Plankton Res.* 6: 295–307.
- FALKOWSKI, P. G., Z. DUBINSKY, AND K. WYMAN. 1985. Growth–irradiance relationships in phytoplankton. *Limnol. Oceanogr.* 30: 311–321.
- HAMMER, U. T. 1978. The saline lakes of Saskatchewan. I. Background and rationale for saline lakes research. *Int. Rev. Gesamten Hydrobiol.* 63: 173–177.
- . 1981. Primary production in saline lakes. A review. *Hydrobiologia* 81: 47–57.
- . 1986. Saline lake ecosystems of the world. Dr. W. Junk Publ., Dordrecht, The Netherlands. 616 p.
- HAMMER, U. T., AND R. C. HAYNES. 1978. The saline lakes of Saskatchewan. II. Locale, hydrography and other physical aspects. *Int. Rev. Gesamten Hydrobiol.* 63: 179–203.
- HARRIS, G. P. 1978. Photosynthesis, productivity and growth: the physiological ecology of phytoplankton. *Ergeb. Limnol.* 10: 1–171.
- HARRISON, W. G., AND T. PLATT. 1980. Variations in assimilation number of coastal marine phytoplankton: effects of environmental co-variables. *J. Plankton Res.* 2: 249–260.
- HAYNES, R. C., AND U. T. HAMMER. 1978. The saline lakes of Saskatchewan. IV. Primary production by phytoplankton in selected saline ecosystems. *Int. Rev. Gesamten Hydrobiol.* 63: 337–351.
- HORNE, A. J., J. C. SANDUSKY, AND C. J. W. CARMIGGELT. 1979. Nitrogen fixation in Clear Lake, California. 3. Repetitive synoptic sampling of the spring *Aphanizomenon* blooms. *Limnol. Oceanogr.* 24: 316–328.
- JELLISON, R., AND J. M. MELACK. 1988. Photosynthetic activity of phytoplankton and its relation to environmental factors in hypersaline Mono Lake, California. *Hydrobiologia* 158: 69–88.
- KIRK, J. T. O. 1977. Use of a quanta meter to measure attenuation and underwater reflectance of photosynthetically active radiation in some inland and coastal southeastern Australian waters. *Aust. J. Mar. Freshwater Res.* 28: 9–21.
- . 1986. Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge. 401 p.
- LEAN, D. R. S., AND B. K. BURNISON. 1979. An evaluation of errors in the ^{14}C method of primary production measurement. *Limnol. Oceanogr.* 24: 917–928.
- MEGARD, R. O., W. S. COMBS, JR., P. D. SMITH, AND A. S. KNOLL. 1979. Attenuation of light and daily integral rates of photosynthesis attained by planktonic algae. *Limnol. Oceanogr.* 24: 1038–1050.
- NUSCH, E. A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. *Ergeb. Limnol.* 14: 14–36.
- RAWSON, D. S., AND J. E. MOORE. 1944. The saline lakes of Saskatchewan. *Can. J. Res. D* 22: 141–201.
- REYNOLDS, C. S. 1984. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge. 384 p.
- ROBERTS, R. D., AND T. ZOHARY. 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *N.Z. J. Mar. Freshwater Res.* 21: 391–399.
- . 1992. The influence of temperature and light on the upper limit of *Microcystis aeruginosa* production in a hypertrophic reservoir. *J. Plankton Res.* 14: 235–247.
- SAUNDERS, G. E., F. B. TRAMA, AND R. W. BACHMAN. 1962. Evaluation of a modified ^{14}C technique for shipboard estimation of photosynthesis in large lakes. *Publ. Great Lakes Res.* Div. 8: 61 p.
- TALLING, J. F. 1965. The photosynthetic activity of phytoplankton in East African lakes. *Int. Rev. Gesamten Hydrobiol.* 50: 1–32.
- . 1982. Utilization of solar radiation by phytoplankton, p. 619–631. *In* C. Helene, M. Charlier, Th. Montenay-Garestier, and G. Laustriat [ed.] Trends in photobiology. Plenum Publ. Corp., New York, NY.
- TIMMS, B. V., U. T. HAMMER, AND J. W. SHEARD. 1986. A study of benthic communities in some saline lakes in Saskatchewan and Alberta, Canada. *Int. Rev. Gesamten Hydrobiol.* 71: 759–777.
- WETZEL, R. G. 1983. Limnology. 2nd ed. Saunders College Publishing, Philadelphia, PA. 767 p.

Life Cycle Patterns, Density, and Frequency of Deformities in *Chironomus* Larvae (Diptera: Chironomidae) over a Contaminated Sediment Gradient

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Along a gradient of contamination in Lake Vossemeer (where contaminated sediments from the River Rhine are deposited out), *Chironomus* cf. *plumosus* larvae occurred at lower population densities and with higher frequencies of deformities as the contamination levels increased. At the least polluted sites, the frequency of larval deformities was elevated only slightly compared with reference levels. At these sites, *C. cf. plumosus* had a bivoltine life cycle and overwintered in the fourth larval instar stage. At moderately contaminated sites, the percentage of prepupal larvae was significantly lower in spring and the development of pupae delayed by 2 wk. At heavily contaminated sites, larval densities were strongly reduced and no pupae were observed at all. Under such conditions, population density seems to be a suitable additional indicator of toxic stress. The frequency of deformed larvae was higher from November to April than from July to October. Maximum frequencies were observed during emergence of the overwintering generation, suggesting that the period between February and April may be the most suitable period for the assessment of this parameter.

Selon un gradient de contamination dans le lac Vossemeer (où se déposent les sédiments contaminés du Rhin), la densité de population des larves de *Chironomus* cf. *plumosus* diminue et la fréquence des difformités augmente au fur et à mesure que le niveau de contamination augmente. Dans les endroits les moins pollués, la fréquence des difformités larvaires n'est que légèrement plus élevée que les valeurs de référence. Dans ces endroits, *C. cf. plumosus* a un cycle biologique bivoltin et passe l'hiver au quatrième stade larvaire. Dans les endroits modérément contaminés, le pourcentage de larves au stade de la prénympe est significativement plus faible au printemps et le développement des chrysalides est retardé de 2 sem. Dans les endroits fortement contaminés, les densités larvaires diminuent considérablement et aucune chrysalide n'a été observée. Dans de telles conditions, la densité de population semble être un indicateur additionnel approprié du stress toxique. La fréquence de larves difformes était plus élevée entre novembre et avril qu'entre juillet et octobre. Les fréquences maximales ont été notées pendant l'émergence de la génération ayant hiverné et l'on peut donc supposer que la période allant de février à avril est la plus appropriée à l'évaluation de ce paramètre.

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Several studies have demonstrated the occurrence of deformities in the mouth parts and antennae of chironomid larvae (Hamilton and Saether 1971; Hare and Carter 1976; Warwick 1980a, 1980b, 1980c, 1985, 1988, 1989, 1990a, 1990b, 1990c, 1991; Wiederholm 1984; Urk and Kerkum 1987; Warwick et al. 1987; Warwick and Tisdale 1988; Pettigrove 1989). The frequencies of deformities were generally higher at localities in which high concentrations of contaminants were present or might be assumed to be present in the environment because of the proximity of waste water discharges (Wiederholm 1984; Urk and Kerkum 1988; Warwick 1988).

Warwick (1988) reviewed current literature and concluded that the potential for using chironomid deformities as an indicator of toxic stress in aquatic systems was excellent. However, a major problem remains the scarcity of experimental data on the induction of deformities by particular contaminants. There

is some experimental evidence that heavy metals cause deformities in the epipharyngeal pecten of *Chironomus* species (Kosalwat and Knight 1987; Guchte and Urk 1989). This presents promising evidence for some specificity of response, but so far, no single contaminant or class of contaminants has been found to which induction of mentum deformities can be directly attributed. Consequently, the nature of information that the frequency of mentum deformities imparts remains unknown; do elevated frequencies merely indicate the presence of toxic stress, or does this variable provide a reliable index of the extent of contamination? Another problem is the lack of baseline data on the frequency of deformities in natural, unstressed populations. Spatial and temporal variations may also render the interpretation of data more difficult (Warwick 1988).

The purpose of this paper is to quantify as much as possible the relationship between the extent of contamination and the frequency of deformities under field conditions. If elevated deformity frequencies are induced by toxic compounds, one might expect that the parameters of population dynamics like growth rate, rate of emergence, and population density would

¹This work of Dr. Geert van Urk has been completed by his colleagues after his sudden death on November 9, 1990. Missed, but remembered by family and colleagues.

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also respond to differences in contamination levels. To that end, we also explored the relationships between the degree of contamination and population dynamics.

In most studies, either the frequencies of deformities were low (<10%) or chironomid population densities were much reduced at the most heavily impacted sites (e.g. Hare and Carter 1976), so that considerable additional effort would be required to establish any measure of seasonal variation in deformity frequencies. In our study area in the Netherlands, however, relatively high population densities in conjunction with high frequencies of deformities are not unusual and provide a unique opportunity to study seasonal variations in the frequency of deformities and the population dynamics of *Chironomus cf. plumosus* larvae.

Materials and Methods

Study Area

The study area is situated near the mouth of the River IJssel, a branch of the River Rhine in the Netherlands. The IJssel flows into the IJsselmeer, a freshwater reservoir created in 1932 by the closure of a dike separating the Zuiderzee, an inland sea, from the North Sea. The study area, now called the Vossemeer, is a long narrow lake between the former coastline and the dike of the Flevoland polder (Fig. 1). At the western end, the lake is about 2–3 m deep. In this area, large amounts of the suspended sediments carried by the IJssel settle out forming a submerged delta, the thickness of which decreases with increasing distance from the mouth of the IJssel. Four sampling stations were sited in this area (Fig. 1). The remainder

of the lake, except for the shipping channel, is shallow. The bottom consists mainly of sand; only at the southern end of the Vossemeer in the shipping channel is mud present, resulting in a bottom composition roughly similar to that at Site 4. Two additional sampling sites (Sites 5 and 6) were chosen in this area. An uncontaminated reference site (R) was chosen in the neighbouring Veluwemeer (52°22'N, 5°38'E) without river influence for purposes of comparison.

The mean grain size of the sediments at Sites 4–6 was considerably greater than at Sites 1–3 (Table 1). Clay size content was noticeably low at all sites. Kjeldahl nitrogen content at Sites 1 and 4 was intermediate between the low values at Sites 5 and 6 and the high values at Sites 2 and 3. The pattern for organic carbon was somewhat similar to Kjeldahl nitrogen, but no discernible pattern was evident for total phosphorus.

The sediments at Sites 1–4 clearly show the effects of pollutant inputs from the Rhine (Table 2). After correction for organic carbon content and particle size (Kooy et al. 1991), almost all persistent organic contaminants and some heavy metals showed a definite clustering of high concentration values around these four sites. Some of the less persistent or volatile substances such as PCB-52 showed a definite concentration gradient, declining in concentration outward from the mouth of the IJssel. The relatively low concentrations of hexachlorobenzene (HCB) and trace metals at Site 1 may be the result of decreased loadings borne by the IJssel in recent years. However, the consistent decrease in concentration in all trace metals from Site 3 toward Site 1 suggests dilution by mineral sediments which are deposited out with the drop in hydraulic energy at the river mouth. The decrease in loadings observed

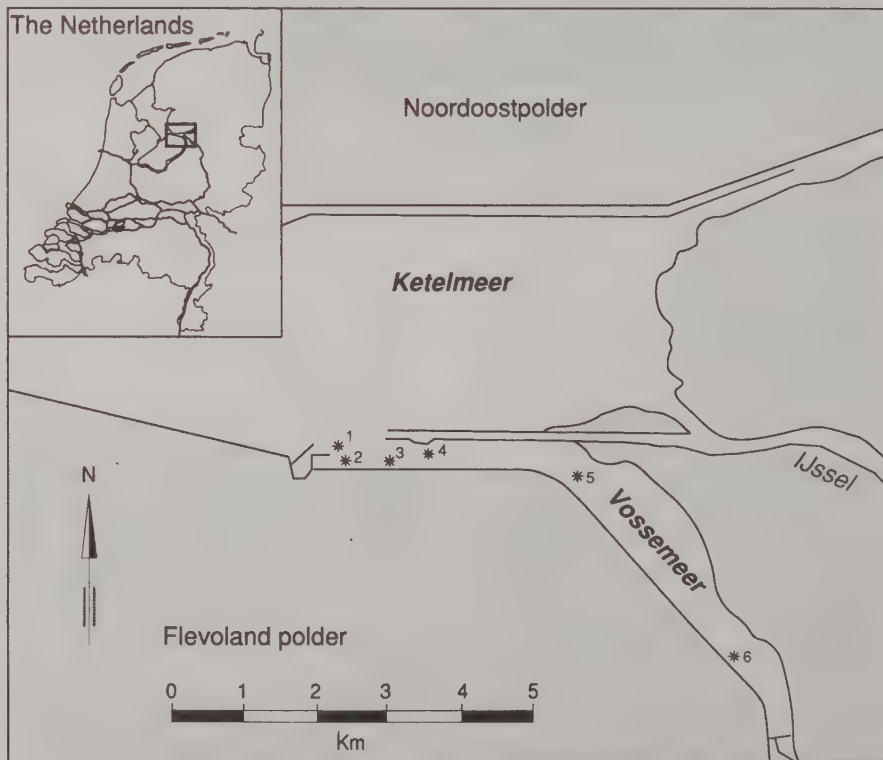


FIG. 1. Location of the study area in The Netherlands. Sampling sites are indicated by the numbers 1–6. Polder = reclaimed land.

TABLE 1. Physical and chemical characteristics of the upper 10 cm of sediment at the sampling sites (1–6 and reference R) in the Vossemeer.

Parameter	Units	1	2	3	4	5	6	R
Dry matter	%	42	35	33	45	48	49	47
Organic carbon	%	3.4	6.1	6.0	2.8	2.2	2.4	2.8
Kjeldahl N	mg/g	14.3	24.8	21.4	9.7	2.1	1.8	0.1
Total P	mg/g	0.6	0.8	0.9	0.5	0.6	0.5	0.5
Sand >60 µm	%	35	13	12	53	48	62	31
Silt 2–60 µm	%	59	81	81	45	50	36	58
Clay <2 µm	%	6	6	7	2	2	2	11

TABLE 2. Concentrations of selected contaminants at the six contaminated sites in the Vossemeer and the uncontaminated reference site (R) in the Veluwemeer.

Parameter	Units	1	2	3	4	5	6	R
Cd	mg/kg	3.4	6.2	6.7	3.1	2.7	1.6	<0.3
Hg	mg/kg	1.1	2.7	3.9	1.3	0.7	0.5	0.2
Pb	mg/kg	85	134	135	71	44	38	8
Cu	mg/kg	64	109	111	55	29	20	7
Fluoranthene	mg/kg	1.8	2.8	2.1	0.4	<0.1	<0.1	<0.1
Benz[a]pyrene	mg/kg	1.2	1.6	1.1	0.4	<0.1	<0.1	<0.1
Indenopyrene	mg/kg	3.1	1.5	0.9	0.5	<0.1	<0.1	<0.1
HCB	µg/kg	47	195	35	11	<1	<1	<1
PCB-52	µg/kg	38	18	16	9	<1	<1	<1
PCB-53	µg/kg	27	41	31	14	<1	<1	<1
PCB-180	µg/kg	14	22	17	9	<1	<1	<1

in recent years for substances such as cadmium does not yet seem to have resulted in any appreciable decrease of sediment concentrations.

Concentrations of heavy metals at Sites 5 and 6, although low, were still higher than background concentrations at Site R. Organic contaminant concentrations were below detection limits at these sites.

Sampling and Analysis

Ten replicate samples were taken at Sites 1–6 fortnightly from February through November 1987 and monthly from March through May 1988 with a 0.0225-m² Ekman grab. At a minimum, the upper 0.15 m of sediment was sampled. Preliminary observations demonstrated that most *Chironomus* larvae occurred at a depth of 0.05–0.10 m; below 0.15 m, very few larvae were found. The sediments were sieved through a sieve of 0.5-mm mesh. The efficiency of the sieving process was assumed to be nearly complete for third- and fourth-instar larvae and for pupae of *Chironomus*. Larvae and pupae were sorted from the sieve by hand and preserved in the field in 80% ethanol.

All larvae were identified and checked for mentum deformities in the laboratory. The larvae were cleared by heating in 40% potassium hydroxide and mounted in glycerol on temporary slides for microscopic examination. All *Chironomus* larvae were identified to species according to Webb and Scholl (1985). To check the larval identifications, about 100 larvae were brought into the laboratory in April 1987 and reared on Tetra-mine[®] fish food. Emerging male imagoes were identified according to Strenzke (1959) and Pinder (1978). Contrary to the larvae, which were identified as *C. muratensis*, the reared adult males were identified as *C. plumosus*. *Chironomus muratensis* is a member of the *plumosus* complex. Because of the resulting uncertainty, the species dealt with in this study has therefore been named *C. cf. plumosus*.

Mentum deformities were scored using the criteria from Warwick (1988), who considers any departure from the normal pattern of the mentum as a deformity. Deformities in other structures of the head capsule, such as the antennae and mandibles, are not considered here. Larvae were assigned to third or fourth instars on the basis of the width of the larval head capsule measured with an ocular micrometer.

Sediment grain size distribution and organic carbon content were determined according to Vierveijzer et al. (1979). Kjeldahl nitrogen and total phosphorus were determined according to Industrial Method 376-75W (Technicon 1975). Heavy metals were determined according to the Dutch standard methods (NEN): cadmium according to NEN 6458, mercury according to NEN 6449, lead according to NEN 6429, and copper according to NEN 6454 (see also Hulscher et al. 1992a). Organic micropollutants were determined according to Hulscher et al. (1992b). For calculating 95% confidence intervals, population densities were transformed using a log-transformation and the frequencies of deformities using an arcsin-square root transformation.

Results

Population Dynamics

Temporal patterns of larval density

In the winter and spring of 1987, only fourth-instar larvae were found at all sites (Fig. 2). Population densities began to decrease gradually after February (Site 6), March (Site 4), or April (Site 5), reaching minimum densities in June when values declined to about 200 ind/m² (Fig. 2). The rate of decrease did not differ significantly at different sites or during different periods, largely because of the great variance in numbers of larvae in the replicate samples from Sites 5 and 6. During the summer, third-instar larvae were found on all sampling dates, but the proportion of third- to fourth-instar larvae remained low

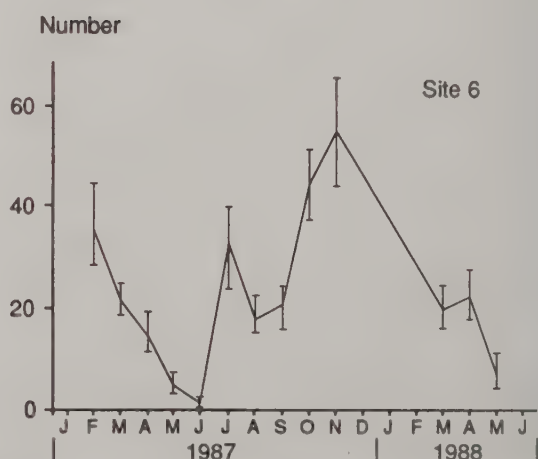
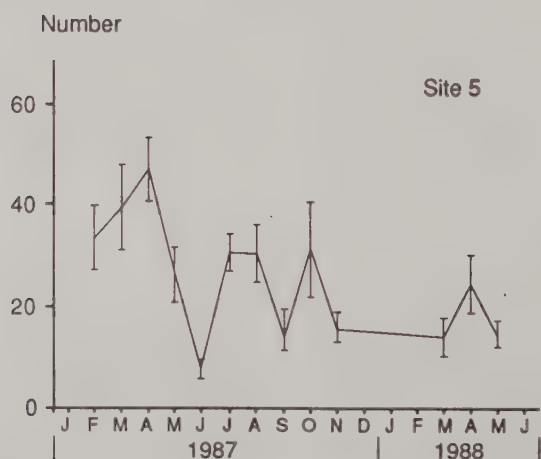
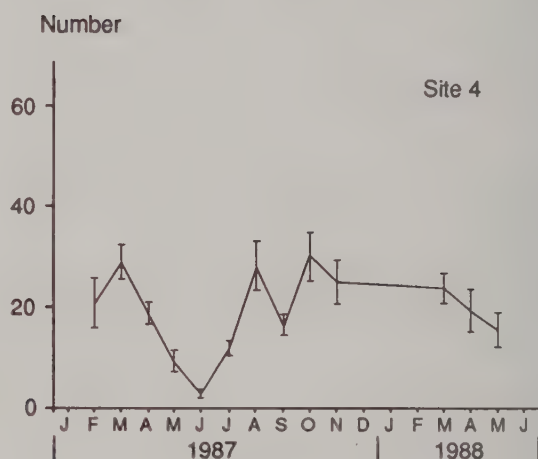
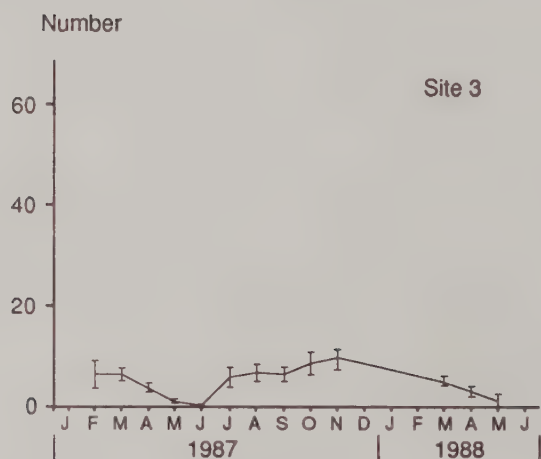
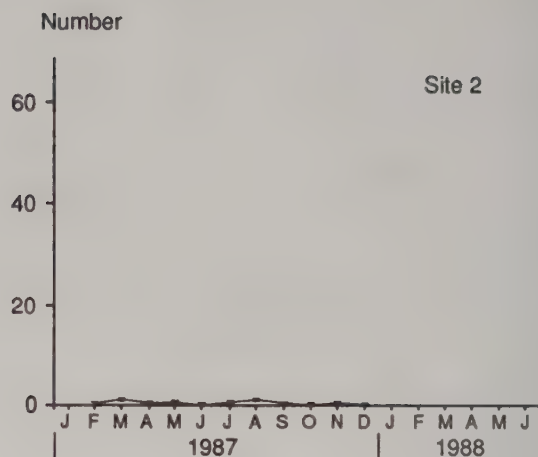
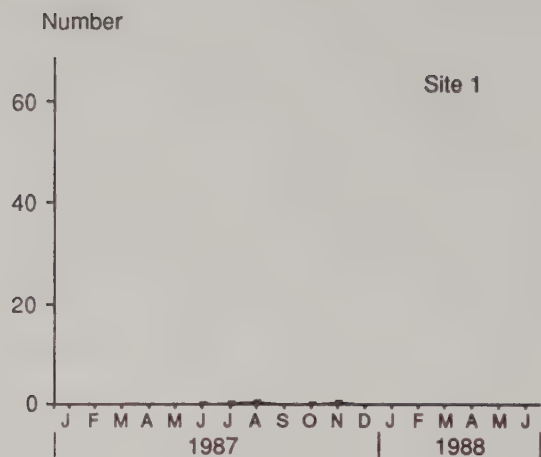


FIG. 2. Average number per grab sample (surface 0.0225 m²) of fourth-instar *C. cf. plumosus* larvae with $\pm 95\%$ confidence limits. Data are averages for each month.

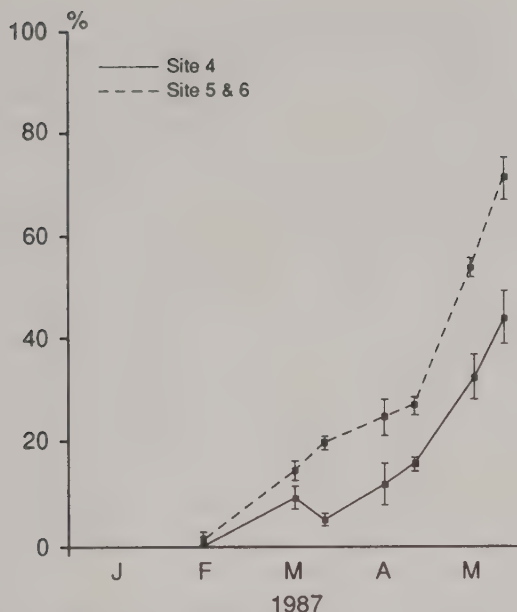


FIG. 3. Frequencies of prepupal fourth-instar *C. cf. plumosus* larvae from January to May 1987.

until September. At this point, larval densities were significantly lower at Sites 4, 5, and 6 than in either August or October. No similar decrease in density was observed at Site 3. The proportion of third-instar larvae continued to decline through October until again, from November 1987 through until the following spring of 1988, only fourth-instar larvae were found.

Spatial patterns of larval density

Larval densities were lowest at Site 1 and significantly higher at each of the Sites 2, 3, and 4 (Wilcoxon $p < 0.01$) (Fig. 2). Densities at Sites 4, 5, and 6 were similar ($p > 0.15$). At Site 1, very few larvae were found compared with Site 2; single larvae were found in less than 50% of the grab samples collected. At Site 3, single larvae were found in nearly 100% of the samples taken.

Frequency of prepupal larvae

Swelling of the thoracic segments is an easily observed external character of larval development. It coincides with phase 9 of fourth-instar larval development (Wülker and Götz 1968). The frequency of prepupal larvae with swollen thoracic segments was always lower at Site 4 than at Sites 5 and 6 on corresponding dates in the spring of 1987 (Fig. 3). This suggests that development at Site 4 is delayed compared with Sites 5 and 6. At Sites 1–3, very few larvae with swollen thoraxes were observed. At these sites, the total number of larvae collected in May was too low to calculate the frequency of prepupal larvae with any degree of precision.

Densities of pupae

Pupae were found at Sites 4, 5, and 6 on all sampling dates from May through September 1987 (Fig. 4). At Site 4, the appearance of pupae was somewhat delayed compared with Sites 5 and 6: the maximum number of pupae found was at Site 4 at the end of May rather than the beginning of May as at the other sites. Pupal densities were much reduced by June. In general, pupal densities were low; seldom did observed densities exceed 50 pupae/m². In the spring of 1988, the pattern

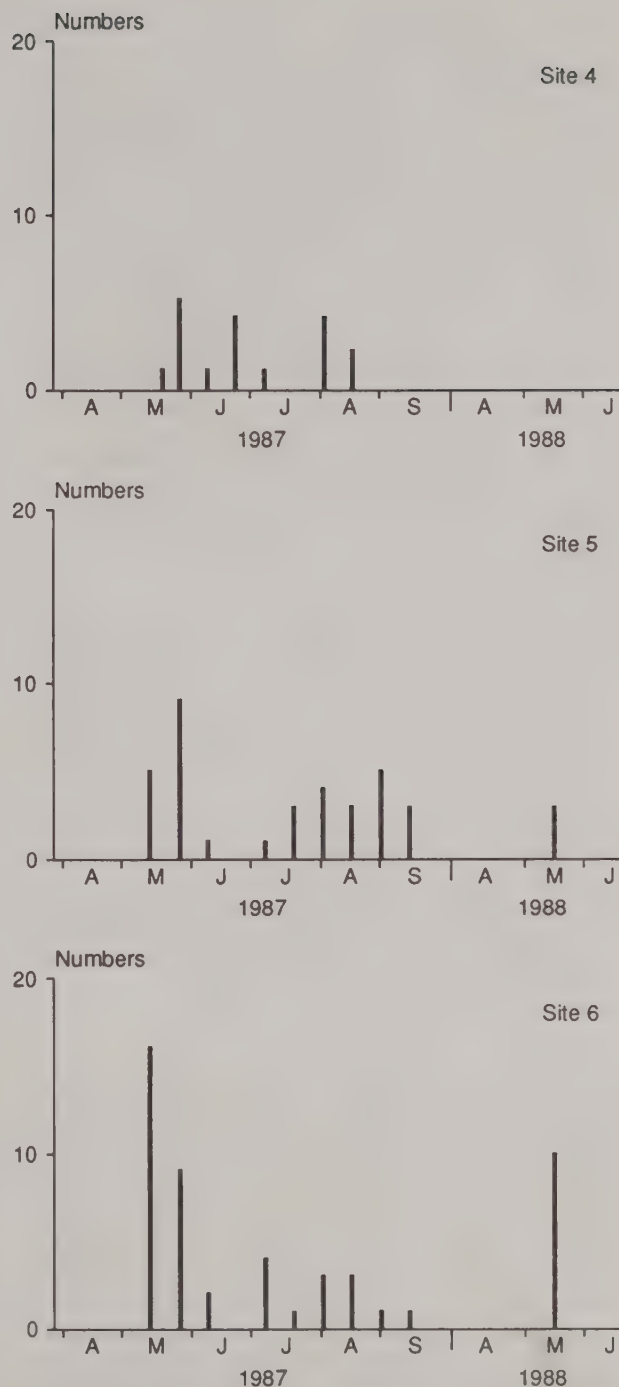


FIG. 4. Total numbers of *C. cf. plumosus* pupae in 10 grab samples (surface 0.225 m²) at Sites 4, 5, and 6.

somewhat differed in that no pupae were found at Site 4, only a few were found at Site 5, and only at Site 6 were numbers of pupae similar to the levels observed in 1987.

No pupae were found at Sites 1, 2, and 3. At Site 3, larval densities were so low that no pupae were to be expected in the samples if the same ratios between the densities of larvae and pupae observed at Sites 5 and 6 were maintained.

Temporal patterns

The frequencies of deformities at the Sites 4, 5, and 6 were lower from midsummer to autumn (July – November) in 1987 than from late winter to spring (February – May and March – May) in 1987 and 1988, respectively (Fig. 5). At each site, frequencies increased between February and April 1987 and between November 1987 and March 1988.

A somewhat different seasonal pattern was observed at Site 3; in the spring of 1987, no increase was observed during the period of emergence, but during the summer the frequency of deformed larvae, although lower than during the preceding winter, did show a definite increase between July and November. In April and May 1988, the frequency of deformed larvae was again higher, attaining frequencies equal to or greater than in the previous spring.

Too few individuals were found at the Sites 1 and 2 to detect any significant differences in temporal distributions. The frequencies of deformities in March, April, and May 1988 were not significantly different from the frequencies for the corresponding months of 1987.

Spatial patterns

The frequencies of deformities were lowest at Sites 5 and 6 (Fig. 5), but levels were still higher than the reference value of 1% at Site R and the values considered to represent background values by most authors (e.g. Warwick 1980a, 1980b, 1985; Wiederholm 1984). Compared with Sites 5 and 6, however, the frequency of deformities was higher at each of Sites 4, 3, and 2 ($p < 0.01$ between pairs of Sites 5, 4, and 3 and $p < 0.05$ between Sites 3 and 2).

The more serious types of deformities also occurred at the sites with the highest frequency of deformities. For example, specimens displaying the Köhn gap, a deformity characterized by a large gap in the mentum which may or may not include the loss of some teeth (Köhn and Frank 1980; Warwick 1988; Warwick and Tisdale 1988), were observed more frequently at sites with high deformity frequencies (Fig. 6).

Discussion

Most authors (e.g. Hilsenhoff 1966; Beattie 1978; Matena 1989) describe *C. plumosus* as bivoltine. Sokolova (1983), who compared data from a wider geographical distribution, concluded that *C. plumosus* might be univoltine in the northern latitudes of the (previously) U.S.S.R. but may have as many as three generations in southern latitudes. In Lake Suwa, Japan, *C. plumosus* produced three generations a year in the littoral zone, but lacked a summer generation in the profundal (Yamagishi and Fukuhara 1971). The *C. cf. plumosus* populations at Sites 4, 5, and 6 in the Vossemeer showed essentially the same pattern of density changes as the populations studied by Matena (1989) and Beattie (1978). This may suggest that at least part of the population is bivoltine, but pupae were found for a long period during summer along with third-instar larvae. This may reflect poor synchronization and the possibility that a part of the population has more than two generations per year or that more than one species is involved. An alternative explanation may be that the cycling of three generations is being phased over 2 yr instead of 1. In these latitudes, the degree/day energy requirements for development may not be sufficient to support two full generations per year, so part of the development period is carried over into the following year, that is, the bivoltine year (Sokolova et al. 1992). It may also be that

population development is being inhibited by contaminant effects at these sites rather than a deficiency in degree/day energy requirements. Contaminants are known to inhibit growth and development (Wentzel et al. 1978) and these sites are not uncontaminated. Although other environmental factors cannot be excluded entirely, the delay in larval development noted at Site 4 supports the contaminant inhibition theory. The percentage of prepupal larvae at Site 4 was lower in the spring and only sporadically present later in the year. The concentration of contaminants in the sediments and water, likely responsible for the higher frequency of deformities and retarded growth at Site 4, apparently was not yet high enough to have a serious impact on population densities.

The increases in the frequency of deformities observed at Sites 4, 5, and 6 between November 1987 and March 1988 also become apparent when data from Urk and Kerkum (1987) for October 1986 are compared with those from the spring of 1987. Since deformities of sclerotized mouthparts may be considered only to occur at the transition between instars and no recruitment, moulting, or emergence occurs during the winter months, these increases could mean any or all of the following: (1) a lower mortality rate for deformed larvae, perhaps because these are less active and consequently less susceptible to predation, (2) a differential emigration of normal larvae from the contaminated areas, or (3) a gradual increase in the catchability of deformed larvae, perhaps because they live nearer the sediment surface. This would imply that in early spring a considerable proportion of the larvae lived below the maximum sampling depth of the Eckman grab (± 20 cm). However, in occasional vertical distribution studies, the vast majority of the larvae was always observed in the upper 10 cm (unpublished data).

The marked rise in frequency observed during emergence of the winter generation may be a mathematical anomaly introduced by the delayed development of deformed larvae. Janssens de Bisthoven and Ollevier (1989) could find no differences in emergence rates between deformed and nondeformed larvae in rearing experiments, but experimental populations held under culture conditions may be exposed to less selective pressure than populations living under natural field conditions.

Both population density and frequency of mentum deformities showed a clear response to the contaminant gradient (Fig. 7). At Sites 5 and 6, the least polluted sites, only the frequency of deformities is increased compared with reference levels. As the severity of pollution increases, prepupal and pupal stages are retarded (Site 4, Fig. 3 and 4) and population densities (Sites 1, 2, and 3) are reduced as well. This sequence of effects is in good accordance with the toxicological principle that sublethal effects very often precede lethal effects and reinforces the contention that gradients of contamination and the frequency of deformities are causally related. The positive correlation between the frequency of mentum deformities and the severity of contamination demonstrated by Warwick (1990a, 1990b, 1991) is further substantiated by these data from a field situation.

With systematic sampling, differences between locations can be recognized more easily. In the Vossemeer, the gradient in deformity frequencies was evident throughout the greater part of the sampling period but was most pronounced from February to April. This suggests that this may be the most suitable period for assessing the frequency of deformities in the menta of *Chironomus* larvae.

Population densities seem to provide an additional indicator of toxic stress. Warwick (1991) found that not only were the populations of individual taxa reduced by contamination, but

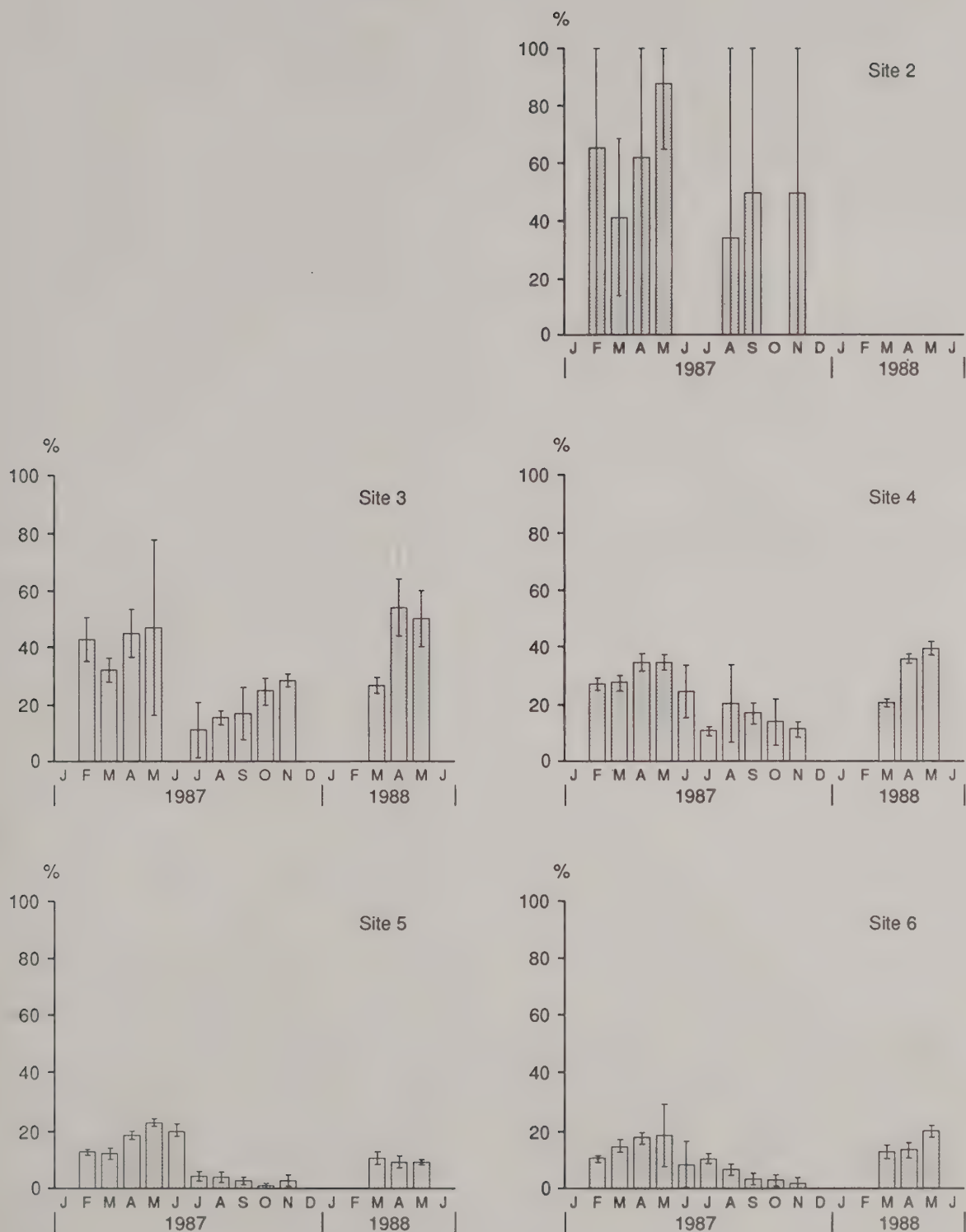


FIG. 5. Temporal patterns of the frequency of deformities at Sites 2-6 (% $\pm$ 95% confidence limits).

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References

- BEATTIE, D. M. 1978. Chironomid populations in the Tjeukermeer. Thesis, University of Leiden, Leiden, The Netherlands.
- GUCHTE, C. VAN DE, AND G. VAN URK. 1989. Discrepancies in the effects of field and artificially contaminated sediments upon midge larvae, p. 574–577. In J. P. Vernet [ed.] Proc. Int. Conf. Heavy Metals in the Environment, Geneva, 1989. Vol. 1. CEP Consultants Ltd., Edinburgh, U.K.
- HAMILTON, A. L., AND O. A. SAETHER. 1971. The occurrence of characteristic deformities in the chironomid larvae of several Canadian lakes. Can. Entomol. 103: 363–368.
- HARE, L., AND J. C. H. CARTER. 1976. The distribution of *Chironomus* (s.s.)? *cucini* (*salinarius* group) larvae (Diptera: Chironomidae) in Parry Sound, Georgian Bay, with particular reference to structural deformities. Can. J. Zool. 54: 2129–2134.
- HILSENHOFF, W. L. 1966. The biology of *Chironomus plumosus* (Diptera: Chironomidae) in Lake Winnebago, Wisconsin. Ann. Entomol. Soc. Am. 59: 465–473.
- HULSCHER, TH. E. M. TEN, G. A. J. MOL, AND F. LÜERS. 1992a. Release of metals from polluted sediments in a shallow lake: quantifying resuspension. Hydrobiologia 235/236: 97–106.
- HULSCHER, TH. E. M. TEN, L. E. VAN DER VELDE, AND W. A. BRUGGEMAN. 1992b. Temperature dependence of Henri's law constants for selected chlorobenzenes, polychlorinated biphenyls and polycyclic aromatic hydrocarbons. Environ. Toxicol. Chem. 11. (In press)
- JANSSENS DE BISTHOVEN, L., AND F. OLLEVER. 1989. Some experimental aspects of sediment stress on *Chironomus* gr. *thummi* larvae (Diptera: Chironomidae). Acta Biol. Debr. Oecol. Hung. 3: 147–155.
- KÖHN, T., AND C. FRANK. 1980. Effects of thermal pollution on the chironomid fauna in an urban channel, p. 187–194. In D. A. Murray [ed.] Chironomidae. Ecology, systematics, cytology and physiology. Pergamon Press, Oxford, U.K.
- KOOP, L. A. VAN DER, D. VAN DE MEENT, C. J. VAN LEEUWEN, AND W. A. BRUGGEMAN. 1991. Deriving quality criteria for water and sediment from the results of aquatic toxicity tests and product standards: application of the equilibrium partitioning method. Water Res. 25: 697–705.
- KOSALWAT, P., AND A. W. KNIGHT. 1987. Chronic toxicity of copper to a partial life cycle of the midge, *Chironomus decorus*. Arch. Environ. Contam. Toxicol. 16: 283–290.
- MATENA, J. 1989. Seasonal dynamics of a *Chironomus plumosus* (L.) (Diptera: Chironomidae) population from a fish pond in southern Bohemia. Int. Revue Gesamten Hydrobiol. 74: 599–610.
- PETTINGROVE, V. 1989. Larval mouthpart deformities in *Procladius paludicola* Skuse (Diptera: Chironomidae) from the Murray and Darling rivers, Australia. Hydrobiologia 179: 111–117.
- PINDER, V. 1978. A key to the adult males of the British Chironomidae (Diptera); the non-biting midges. Freshwater Biol. Assoc. Sci. Publ. No. 37.
- SOKOLOVA, N. Y. 1983. The non-biting midge *Chironomus plumosus* (L.) (Diptera: Chironomidae). Izd. Nauka Mosk. (In Russian)
- SOKOLOVA, N. Y., A. V. PALY, AND E. I. IZVEKOVA. 1992. Biology of *Chironomus piger* Str. (Diptera: Chironomidae) and its role in the self-purification of a river. Neth. J. Aquat. Ecol. 26. (In press)
- STRENZKE, K. 1959. Revision der Gattung *Chironomus* Meigen 1. Die Imagines von 15 norddeutschen Arten und Underarten. Arch. Hydrobiol. 56: 1–42.
- TECHNICON. 1975. B.D.-40 Block digester manual, TA 4-0323-00. Technicon Instruments Corporation, Tarrytown, NY.
- URK, G. VAN, AND F. C. M. KERKUM. 1987. Chironomid mortality after the Sandoz accident and deformities in *Chironomus* larvae due to sediment pollution in the Rhine. Aqua 4: 191–196.
1988. Bottom fauna of polluted Rhine sediments, p. 1405–1407. In K. Wolf, W. J. van den Brink, and F. J. Colon [ed.] Contaminated soil '88. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- VIERVEIJER, H. C., A. LEPELAAR, AND J. DIJKSTRA. 1979. Analyse-methoden voor grond, rioolslib, gewas en vloeistof. Rep. Inst. Bodemvruchtbaarheid, Groningen. (In Dutch)
- WARWICK, W. F. 1980a. Palaeolimnology of the Bay of Quinte, Lake Ontario: 2800 years of cultural influence. Can. Bull. Fish. Aquat. Sci. 206: 117 p.
- 1980b. Chironomidae (Diptera) responses to 2800 years of cultural influence: a palaeolimnological study with special reference to sediment-

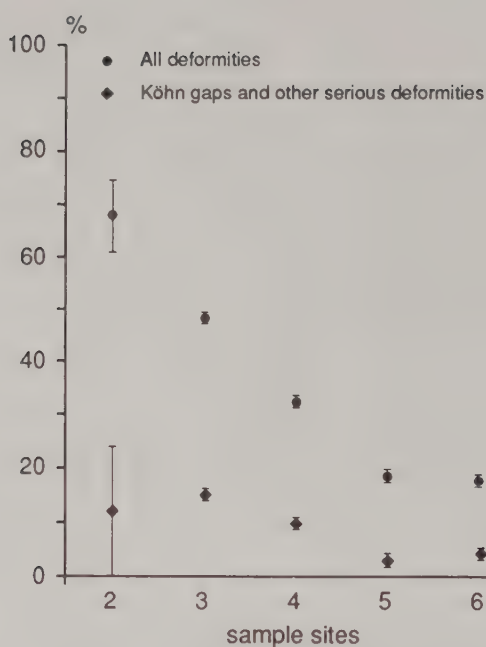


FIG. 6. Frequency of total deformities and the more serious types of deformities (e.g. the Köhn gap) at Sites 3–6. Data are averages over the period February – April 1987 (% $\pm$ 95% confidence limits).

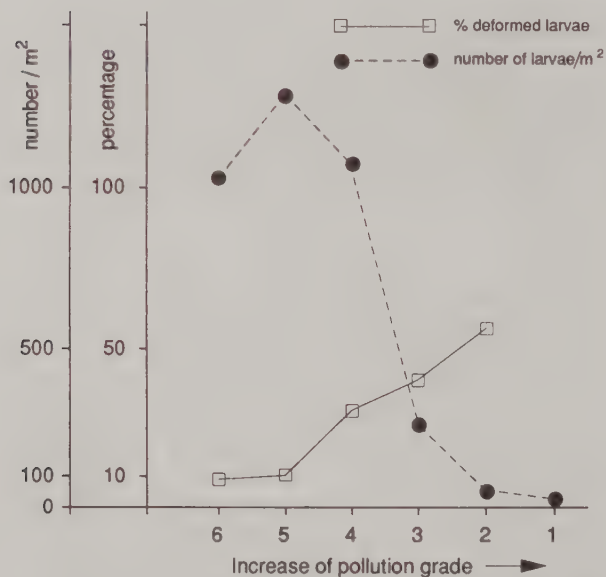


FIG. 7. Relationship between the degree of pollution, population densities, and the frequencies of deformities over all six sites. Data are averages for the period February – April 1987.

many components of chironomid communities were eliminated entirely from their environment. In water bodies where *Chironomus* forms a major component of the macrobenthos, it may in future become possible to predict the effects of toxic stress on other trophic levels.

- tation, eutrophication and contamination processes. *Can. Entomol.* 112: 1193–1238.
- 1980c. Pasqua Lake, southeastern Saskatchewan: a preliminary assessment of trophic status and contamination based on the Chironomidae (Diptera), p. 255–267. *In* D. A. Murray [ed.] Chironomidae: ecology, systematics, cytology and physiology. Pergamon, Oxford, U.K.
1985. Morphological abnormalities in Chironomidae (Diptera) larvae as measures of toxic stress in freshwater ecosystems: indexing antennal deformities in *Chironomus* Meigen. *Can. J. Fish. Aquat. Sci.* 42: 1881–1914.
1988. Morphological deformities in Chironomidae (Diptera) larvae as biological indicators of toxic stress, p. 281–320. *In* M. S. Evans [ed.] Toxic contaminants and ecosystem health: a Great Lakes focus. Wiley & sons, New York, NY.
1989. Morphological deformities in larvae of *Procladius* Skuse (Diptera: Chironomidae) and their biomonitoring potential. *Can. J. Fish. Aquat. Sci.* 46: 1255–1271.
- 1990a. The use of morphological deformities in chironomid larvae for biological effects monitoring. *Environ. Can. Inland Waters Dir. Sci. Publ. Ser. No.* 173: 1–34.
- 1990b. Morphological deformities in Chironomidae (Diptera) larvae from the Lac St. Louis and Laprairie basins of the St. Lawrence River. *J. Great Lakes Res.* 16: 185–208.
- 1990c. Chironomidae (Diptera) responses to contaminants in the St. Lawrence River, p. 525–528. *In* J. Barceló [ed.] Environmental contamination. Fourth International Conference, 1–4 October 1990, Barcelona, Spain. CEP Consultants Ltd., Edinburgh, U.K.
1991. Indexing deformities in ligulae and antennae of *Procladius* larvae (Diptera: Chironomidae): application to contaminant-stressed environments. *Can. J. Fish. Aquat. Sci.* 48: 1151–1166.
- WARWICK, W. F., J. FITCHKO, P. M. MCKEE, D. R. HART, AND A. J. BURT. 1987. The incidence of deformities in *Chironomus* spp. from Port Hope Harbour, Lake Ontario. *J. Great Lakes Res.* 13: 88–92.
- WARWICK, W. F., AND N. A. TISDALE. 1988. Morphological deformities in *Chironomus*, *Cryptochironomus* and *Procladius* larvae (Diptera: Chironomidae) from two differentially stressed sites in Tobin Lake, Saskatchewan. *Can. J. Fish. Aquat. Sci.* 45: 1123–1144.
- WEBB, C. J., AND A. SCHOLL. 1985. Identification of larvae of European species of *Chironomus* Meigen (Diptera: Chironomidae) by morphological characters. *Syst. Entomol.* 10: 353–372.
- WENTSEL, R., A. MCINTOSH, AND W. P. MCCAFFERTY. 1978. Emergence of the midge *Chironomus tentans* when exposed to heavy metal contaminated sediment. *Hydrobiologia* 57: 195–196.
- WIEDERHOLM, T. 1984. Incidence of deformed chironomid larvae (Diptera: Chironomidae) in Swedish lakes. *Hydrobiologia* 109: 243–249.
- WÜLKER, W., AND P. GÖTZ. 1968. Die Verwendung der Imaginalschelben zur Bestimmung des Entwicklungszustandes von *Chironomus*-Larven (Dipt.) Z. Morphol. Oecol. Tiere 62: 363–388.
- YAMAGISHI, H., AND H. FUKUHARA. 1971. Ecological studies on chironomids in Lake Suwa. Population dynamics of *Chironomus plumosus* L. and *Spaniatoma akamusi* Tokunaga. *Oecologia* 7: 309–327.

Genetic Control of Juvenile Life History Pattern in Chinook Salmon (*Oncorhynchus tshawytscha*)

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To investigate the genetic basis for the difference in photoperiod responses between juvenile ocean-type and stream-type chinook salmon (*Oncorhynchus tshawytscha*), we conducted two crossing experiments and exposed the progeny to either a short- or long-day photoperiod for 10 wk from the time of first feeding. The first experiment examined the photoperiod response of pure and reciprocal crosses among red- and white-fleshed Quesnel River (stream-type) chinook salmon. The second experiment tested the photoperiod response of pure and reciprocal crosses between Quesnel River (stream-type) and Conuma River (ocean-type) chinook salmon. In both experiments, Quesnel chinook salmon fry (both red and white fleshed) sustained a high growth rate and developed a high degree of seawater adaptability only when exposed first to a short-day photoperiod for 10 wk and then to a long-day photoperiod. In contrast, the Conuma River chinook salmon grew rapidly and developed the increased seawater adaptability characteristic of smolts when reared on either photoperiod regime. Reciprocal Conuma–Quesnel hybrids displayed the ocean-type pattern of development, indicating that the photoperiod-independent phenotype is dominant and not under maternal control.

Afin d'étudier les fondements génétiques des différences sur le plan de la réaction à la photopériode observées entre le jeune saumon quinnat (*Oncorhynchus tshawytscha*) des océans et celui des cours d'eau, nous avons effectué des expériences de croisement et avons exposé la progéniture à une photopériode à phase diurne longue ou courte pendant 10 sem à partir de la première prise de nourriture. Dans la première expérience, nous avons étudié la réaction à la photopériode de croisements purs et réciproques de saumon quinnat à chair rouge et à chair blanche de la rivière Quesnel (saumon de cours d'eau). Dans la deuxième expérience, nous avons étudié la réaction à la photopériode de croisements purs et réciproques entre le saumon quinnat de la rivière Quesnel (saumon de cours d'eau) et de la rivière Conuma (saumon océanique). Dans les deux expériences, le jeune saumon quinnat de Quesnel (à chair rouge ou à chair blanche) a connu un taux de croissance élevé et a manifesté une grande faculté d'adaptation à l'eau de mer uniquement lorsqu'il était d'abord exposé à une photopériode à phase diurne courte pendant 10 sem, puis à une photopériode à phase diurne longue. En revanche, le saumon quinnat de la rivière Conuma s'est développé rapidement et a acquis la faculté d'adaptation à l'eau de mer accrue caractéristique des smolts, quel que soit le régime de photopériode dans lequel il était élevé. Les hybrides issus des croisements réciproques Conuma–Quesnel ont manifesté le type de développement océanique, ce qui indique que le phénotype indépendant de la photopériode est dominant et n'est pas déterminé par le génome maternel.

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Chinook salmon (*Oncorhynchus tshawytscha*) exhibit two major life history patterns with different lengths of freshwater residence: "ocean-type" juveniles migrate to sea as underyearlings whereas "stream-type" juveniles reside in fresh water for one or more years before developing into smolts and migrating to the sea (Healey 1983, 1991; Carl and Healey 1984; Taylor 1990a). The parr-smolt transformation characterized by numerous morphological and physiological changes including rapid growth and increased ability to adapt to seawater is synchronized by the annual photoperiod cycle in various salmonids (Hoar 1988; Clarke 1989). Recently, we found a difference in photoperiod response between stream- and ocean-type chinook salmon fry. Stream-type (Kitsumkalum River) fry exposed to a short-day photoperiod for 2 mo followed by exposure to a long-day photoperiod responded with the accelerated growth and development of increased seawater adaptability characteristic of smolts whereas those exposed to a long-day photoperiod from the time of first feeding remained

parrlike with slower growth and poor seawater adaptability (Clarke et al. 1989). In contrast, ocean-type (Big Qualicum River) fry grew well and adapted readily to seawater after either photoperiod treatment (Clarke et al. 1989).

The present investigation was undertaken to determine the genetic basis for variation among chinook salmon populations with respect to photoperiod control of smolting. Two experiments were conducted. The first was conducted to determine whether red- and white-fleshed chinook salmon from the Quesnel River have different responses to long- and short-day treatment. Although the determination of red and white flesh colour in Quesnel River chinook salmon is under genetic control (Withler 1986), it is not known whether the two phenotypes belong to a single polymorphic population or to two subpopulations with restricted gene interchange. The second experiment tested the photoperiod responses of crosses among Quesnel River (stream-type) and Conuma River (ocean-type) chinook salmon.

Materials and Methods

Experiment 1

Adult chinook salmon captured in the Quesnel River, a tributary of the upper Fraser River, were classified as "red" (R) or "white" (W) on the basis of external skin colour and flesh colour (Withler 1986). Gametes were collected from each of four white females, white males, red females, and red males. Eggs and milt were transported separately to the Rosewall Creek Hatchery on Vancouver Island and then fertilized in an 8×8 factorial design to produce four cross types: $W \times W$, $W \times R$, $R \times R$, and $R \times W$. Thus, each cross type included 16 families of fish. In late January, fry were removed from the incubation trays and transported to the Pacific Biological Station. A group of 80 fry from each cross type was placed in a 197-L tank in each of two separate rooms. Fry in one room received a short-day photoperiod (9.5 h light : 14.5 h dark) and in the second a long-day one (14.5 h light : 9.5 h dark) which was equivalent to natural daylength plus civil twilight on April 10. These photoperiods were maintained until April 10 and then both rooms received a simulated natural photoperiod cycle increasing from 14.5 h light : 9.5 h dark. Fry were hand-fed with Ewos starter diet initially, and then White Crest feed was delivered using automatic feeders throughout the daylight hours at 1.5 times the level recommended by the manufacturers, with pellet size adjusted as fish grew. Water temperature was held at 10°C until June 23 when the water supply was switched to 29‰ seawater at 14°C. The fish were maintained until November 16. Weight and length measurements were made on all fish in each tank on March 5, April 2, April 30, May 28, and June 19 (fresh water) and July 2, July 30, and August 14 (salt water). Samples of 10 fish per tank were removed for 24-h seawater challenge tests (Blackburn and Clarke 1987) on April 22, May 14, June 8, and June 22. The following analysis of variance (ANOVA) model was used to examine variation in weight, length, growth rate, and plasma sodium levels for the fish in this experiment:

$$Y_{ijk} = \mu + P_i + C_j + P \cdot C_{ij} + e_{ijk}$$

where Y_{ijk} is the individual measurement, μ is the overall mean, P_i is the fixed effect of photoperiod ($i = 1, 2$), C_j is the fixed effect of cross type ($j = 1, 4$), $P \cdot C_{ij}$ is the interaction between photoperiod and cross type, and e_{ijk} is the error term of the k th observation in subgroup ijk .

Experiment 2

Gametes were collected from adult chinook salmon captured in the Quesnel River (Q) and at the Conuma River (C) enhancement facility on the west coast of Vancouver Island. Eggs and milt were taken from nine males and nine females of each stock. Gametes from the Quesnel River population were obtained from both red and white males and females. The gametes were transported separately to the Pacific Biological Station where they were fertilized in three sets of 6×6 crosses to produce four cross types: $C \times C$, $C \times Q$, $Q \times C$, and $Q \times Q$. Within each of the three sets, nine families of each cross type were created (three males by three females). This was done to ensure an adequate sample of the genetic variation within the two wild populations so that differences among cross types are more likely to reflect differences between populations rather than among individuals within populations. Family identity was not maintained past incubation, but the group of fry representing each replicate group of each cross type consisted of equal num-

bers of fry from all 27 families (three sets by nine crosses) of that cross type. On January 18, fry were removed from the incubation trays and 150 fry from each cross type were placed in each of two replicate 197-L tanks in each of two separate rooms. Fry in one room received a short-day photoperiod (10 h light : 14 h dark) and in the second a long-day photoperiod (14 h light : 10 h dark). The photoperiod levels were held constant until April 1 when both rooms were switched to a simulated natural photoperiod increasing from 14 h light : 10 h dark. The actual daylengths were adjusted slightly from those in experiment 1 in order that the long-day photoperiod would coincide with natural daylength plus civil twilight on April 1. Water temperature was 11°C until June 23 when the incoming water supply was switched to 29‰ seawater at 14°C.

On March 29, 20 fish in each tank were implanted intraperitoneally with PIT tags (Destron Identification Devices, Inc.) in order that individual growth rates could be determined. Weight measurements on the PIT-tagged fish were taken on April 5, June 22, June 24, and November 16 and were used to calculate the instantaneous freshwater (April 5 – June 22) and seawater (June 24 – November 16) growth rates. The following ANOVA model was used to examine variability in growth rates in fresh and salt water as well as weight at the end of the experiment on November 16:

$$Y_{ijkl} = \mu + P_i + C_j + P \cdot C_{ij} + R_{ijk} + e_{ijkl}$$

where Y_{ijkl} is the individual measurement, μ is the overall mean; P_i is the fixed effect of photoperiod ($i = 1, 2$), C_j is the fixed effect of cross type ($j = 1, 4$), $P \cdot C_{ij}$ is the interaction between photoperiod and cross type, R_{ijk} is replicate of subgroup ijk ($k = 1, 2$), and e_{ijkl} is the error term of the l th observation in subgroup ijk .

Samples of five fish from each tank were removed for 24-h seawater challenge tests in March, April, and May; a sample of 12 fish was taken from each tank for a seawater challenge test on 19 June, just prior to transfer of the remaining fish to seawater. Fish from replicate tanks were combined for the challenge tests, so variability in the plasma sodium levels was examined using the following ANOVA model:

$$Y_{ijkl} = \mu + P_i + C_j + D_k + P \cdot C_{ij} + e_{ijkl}$$

where Y_{ijkl} is the individual sodium measurement, μ is the overall mean; P_i is the fixed effect of photoperiod ($i = 1, 2$), C_j is the fixed effect of cross type ($j = 1, 4$), D_k is the fixed effect of sampling date ($k = 1, 4$), $P \cdot C_{ij}$ is the interaction between photoperiod and cross type, and e_{ijkl} is the error term of the l th observation in subgroup ijk .

Results

Experiment 1

Growth in weight was similar among all four flesh colour groups under both photoperiod treatments during February, March, and April (Fig. 1). During this period, the mean specific growth rate for weight did not vary significantly with photoperiod treatment ($F_{1,3} = 2.87$, $p > 0.01$) (Table 1). However, during May and June, fish previously exposed to the short-day photoperiod grew 1.6 times as fast ($F_{1,3} = 101.82$, $p < 0.005$) as did the fish exposed to long-day photoperiod (data not shown). During the final 6 wk in seawater, the short-day fish grew 2.7 times as fast ($F_{1,3} = 60.36$, $p < 0.005$) as the long-day fish (Table 1). As a result, the short-day fish had a significantly greater fork length ($F_{1,3} = 58.96$, $p < 0.005$),

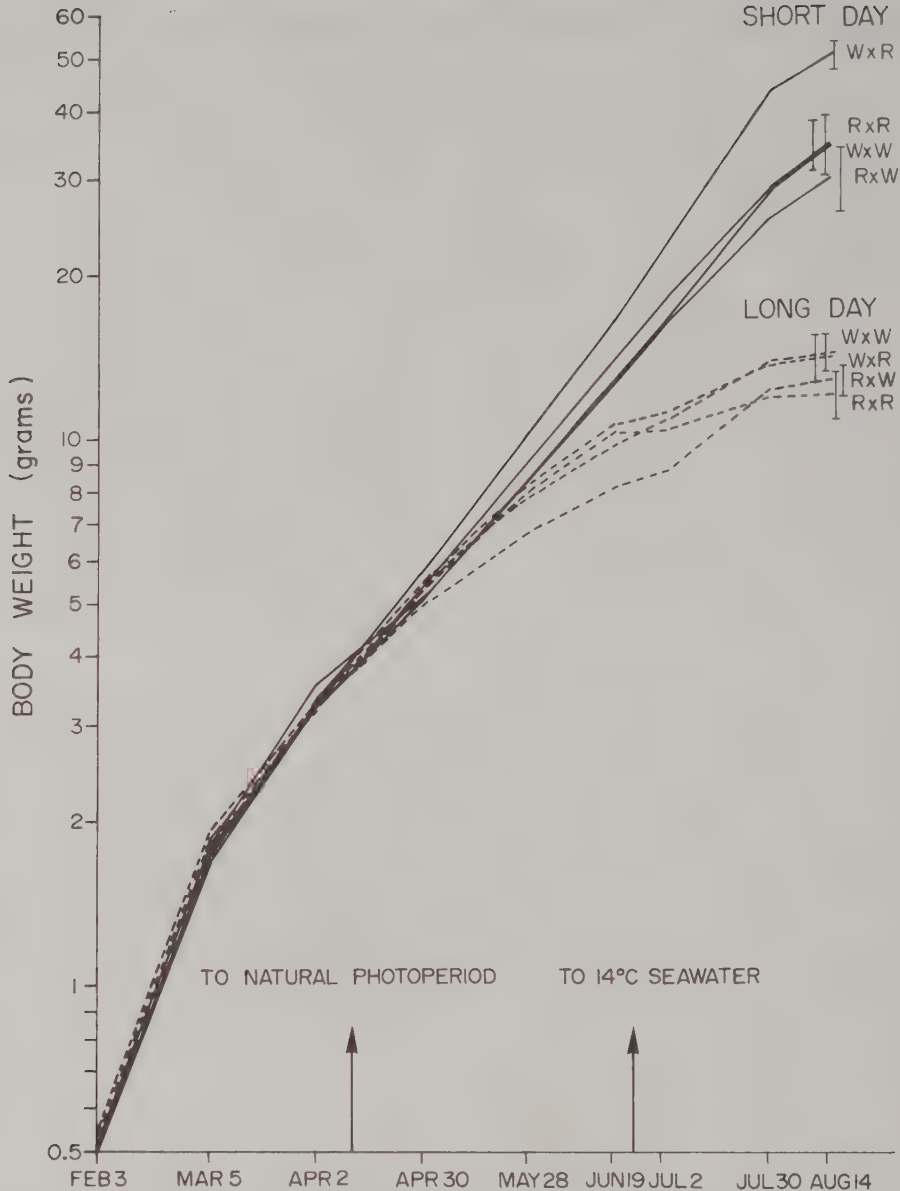


FIG. 1. Influence of long- and short-day photoperiod treatments for 10 wk from the time of first feeding on subsequent growth in fresh and salt water of progeny of crosses among red- (R) and white-fleshed (W) stream-type chinook salmon from the Quesnel River.

weight ($F_{1,3} = 31.05$, $p < 0.025$), and condition factor ($F_{1,3} = 41.03$, $p < 0.01$) at the end of the experiment on August 14 (Table 1). In contrast, there were no significant differences due to cross type with respect to fork length ($F_{3,3} = 1.4$, $p > 0.25$), weight ($F_{3,3} = 1.25$, $p > 0.25$), or condition factor ($F_{3,3} = 3.28$, $p > 0.1$) on August 14. As with the growth responses, seawater adaptability as judged by plasma sodium levels after 24-h seawater challenge tests was significantly affected by photoperiod ($F_{1,3} = 45.23$, $p < 0.01$) but not by cross type ($F_{3,3} = 1.13$, $p > 0.25$) (Table 2). On the one hand, the long-day fish did not regulate plasma sodium concentrations below $170 \text{ mmol} \cdot \text{L}^{-1}$ during any of the challenge tests. On the other hand, the short-day fish regulated plasma sodium levels below $170 \text{ mmol} \cdot \text{L}^{-1}$ during May and

June, indicating a high degree of seawater adaptability. This difference in seawater adaptability was not related to size variation between photoperiod treatments, since there was no significant correlation between fork length and plasma sodium concentration in the May and June tests for either the long-day ($r = 0.07$, $n = 118$) or short-day fish ($r = -0.05$, $n = 121$). Mortality after transfer of all fish to seawater (June 23 – August 14) was less than 5% with the exception of the $R \times R$ cross which lost 7% in the short-day group and 18% in the long-day group.

Experiment 2

Growth in weight varied little among the Quesnel, Conuma, and hybrid groups of chinook salmon fry on either photoperiod

TABLE 1. Fork length, wet weight, and condition factor on August 14 of Quesnel River chinook salmon of four cross types under two photoperiods. Values are given as mean $\pm$ SE. The specific growth rate (G) for weight in fresh water (FW) from February 3 to April 30 and in seawater (SW) from July 2 to August 14 is also given by cross type and photoperiod.

Photoperiod	Cross type (♂ × ♀)	Fork length (cm)	Weight (g)	Condition factor (100·wt·L ⁻³)	G (% bw·d ⁻¹)	
					FW	SW
Long day	R × R	10.2 $\pm$ 0.1	12.2 $\pm$ 0.6	1.13 $\pm$ 0.02	2.73	0.25
	R × W	10.2 $\pm$ 0.1	12.9 $\pm$ 0.4	1.18 $\pm$ 0.01	2.55	0.90
	W × R	10.5 $\pm$ 0.1	14.2 $\pm$ 0.7	1.21 $\pm$ 0.02	2.78	0.58
	W × W	10.5 $\pm$ 0.1	14.4 $\pm$ 0.6	1.22 $\pm$ 0.01	2.66	0.68
	Mean	10.3	13.4	1.19	2.73	0.60
Short day	R × R	13.8 $\pm$ 0.3	34.8 $\pm$ 2.1	1.29 $\pm$ 0.02	2.76	1.53
	R × W	13.1 $\pm$ 0.3	30.1 $\pm$ 2.0	1.26 $\pm$ 0.03	2.65	1.46
	W × R	15.5 $\pm$ 0.2	50.9 $\pm$ 1.8	1.34 $\pm$ 0.02	2.86	1.86
	W × W	13.7 $\pm$ 0.2	34.6 $\pm$ 1.7	1.32 $\pm$ 0.04	2.65	1.72
	Mean	14.0	37.6	1.30	2.68	1.64

TABLE 2. Plasma sodium concentration (mmol·L⁻¹) after a 24-h seawater challenge of Quesnel River chinook salmon of four cross types under two photoperiods. Values are given as mean $\pm$ SE. *n* = 10 in each test.

Photoperiod	Cross type (♂ × ♀)	Test date				Cross mean
		April 23	May 15	June 9	June 23	
Long day	R × R	176.4 $\pm$ 1.9	183.0 $\pm$ 1.6	189.2 $\pm$ 3.7	179.6 $\pm$ 1.3	182.0
	R × W	170.2 $\pm$ 2.0	173.6 $\pm$ 2.2	179.7 $\pm$ 1.9	177.9 $\pm$ 1.0	175.3
	W × R	182.3 $\pm$ 4.5	186.9 $\pm$ 3.2	185.3 $\pm$ 1.9	185.0 $\pm$ 0.9	185.0
	W × W	173.6 $\pm$ 1.6	176.6 $\pm$ 2.0	177.3 $\pm$ 2.9	179.3 $\pm$ 1.7	176.7
	Mean					179.7
Short day	R × R	177.1 $\pm$ 1.6	159.9 $\pm$ 1.4	154.8 $\pm$ 0.7	164.1 $\pm$ 0.5	164.0
	R × W	177.8 $\pm$ 2.9	161.1 $\pm$ 1.5	156.4 $\pm$ 0.7	163.6 $\pm$ 0.8	164.7
	W × R	170.4 $\pm$ 2.6	165.8 $\pm$ 1.7	160.2 $\pm$ 1.7	166.2 $\pm$ 1.5	165.6
	W × W	171.4 $\pm$ 2.6	168.9 $\pm$ 4.1	157.6 $\pm$ 0.9	162.2 $\pm$ 1.6	165.0
	Mean					164.8

during February and March. The sampling on April 5 at the beginning of the simulated natural photoperiod treatment revealed no significant differences in body weight due to the initial photoperiod treatment ($F_{1,3} = 2.8$, $p > 0.1$) or cross type ($F_{3,3} = 4.6$, $p > 0.1$). Within cross types, specific growth rates for fish in fresh water from April 5 to June 22 varied by 5% or less between photoperiods, except for the Q × Q cross which grew 50% faster after the short-day treatment than after the long-day treatment (Table 3). This difference in growth rate changed the ranking of the Q × Q cross from first under the short-day photoperiod to last under the long-day photoperiod. As a consequence, ANOVA revealed a significant interaction between cross type and photoperiod ($F_{3,8} = 45.62$, $p < 0.001$). Although the mean growth rate for Q × Q fish in the long-day treatment was 1.3%·d⁻¹, there were two exceptional fish which grew at 2.4 and 2.1%·d⁻¹, respectively, and were the only survivors in seawater at the end of the experiment.

Mortality in fresh water was less than 1% in all tanks, but within 3 wk after transfer to seawater, the long-day-treated Q × Q cross suffered 75% mortality, compared with a 6% loss in the short-day Q × Q cross. Only the two fish previously mentioned out of 35 (6%) of the long-day-treated Q × Q cross survived until November. For the other crosses exposed to the long-day photoperiod, cumulative mortality during the entire

period in seawater averaged 14%; for all crosses exposed to the short-day photoperiod, it was 19%.

Mean specific growth rate for the fish surviving in seawater until November did not vary significantly with photoperiod ($F_{1,3} = 0.03$, $p > 0.8$), cross type ($F_{3,3} = 2.99$, $p > 0.1$), or replicate tanks ($F_{7,193} = 1.78$, $p > 0.05$) but the interaction between cross type and photoperiod approached significance at the 5% level ($F_{3,7} = 4.23$, $p = 0.053$) due primarily to the C × C fish (see Table 3). Despite the lack of statistically significant differences in growth rate in seawater, weight at the end of the experiment varied significantly by photoperiod ($F_{1,3} = 32.4$, $p < 0.01$) and cross type ($F_{3,3} = 56.2$, $p < 0.001$) due to the cumulative effect of divergent growth rates in fresh and in salt water (Table 4); there was no interaction between photoperiod and cross type ($F_{3,7} = 0.5$, $p > 0.1$) nor any effect due to replicate tanks ($F_{7,193} = 1.0$, $p > 0.1$). Final length showed the same trends as weight (Table 4). Reciprocal hybrids were intermediate in length and weight between the pure strains. Condition factor did not vary with photoperiod ($F_{1,3} = 2.1$, $p > 0.1$) but there was a significant effect of cross type ($F_{3,3} = 57.1$, $p < 0.005$) and of replicate tanks ($F_{7,193} = 2.3$, $p < 0.05$). Performance in the 24-h seawater challenge tests was poor in all groups during March and April but improved in most groups during May and

TABLE 3. Specific growth rate (G) for weight of crosses among tagged Quesnel (stream-type) and Conuma River (ocean-type) chinook salmon from April 5 to June 22 in fresh water (FW) at 11°C and from June 24 to November 16 in seawater (SW) at 14°C. Values are given as mean $\pm$ SE.

Photoperiod	Cross type ($\delta \times \varphi$)	n	G_{FW} (%bw·d ⁻¹)	n	G_{SW} (%bw·d ⁻¹)
Short day	C $\times$ C	35	1.44 $\pm$ 0.04	26	1.37 $\pm$ 0.04
	C $\times$ Q	33	1.73 $\pm$ 0.05	31	1.66 $\pm$ 0.02
	Q $\times$ C	35	1.77 $\pm$ 0.03	32	1.54 $\pm$ 0.03
	Q $\times$ Q	25	1.97 $\pm$ 0.05	22	1.63 $\pm$ 0.06
	Mean		1.71		1.55
Long day	C $\times$ C	34	1.50 $\pm$ 0.04	28	1.57 $\pm$ 0.03
	C $\times$ Q	37	1.68 $\pm$ 0.05	32	1.74 $\pm$ 0.03
	Q $\times$ C	37	1.86 $\pm$ 0.04	35	1.42 $\pm$ 0.03
	Q $\times$ Q	35	1.31 $\pm$ 0.05	2	1.53 $\pm$ 0.09
	Mean		1.60		1.57

TABLE 4. Fork length, wet weight, and condition factor on November 16 of crosses among Conuma (ocean-type) and Quesnel River (stream-type) chinook salmon in seawater. Values are given as mean $\pm$ SE (replicate tanks combined).

Photoperiod	Cross type ($\delta \times \varphi$)	n	Fork length (cm)	Weight (g)	Condition factor (100·wt·L ⁻³)
Short day	C $\times$ C	26	18.9 $\pm$ 0.4	88.3 $\pm$ 5.2	1.27 $\pm$ 0.02
	C $\times$ Q	31	21.0 $\pm$ 0.3	129.9 $\pm$ 6.2	1.36 $\pm$ 0.02
	Q $\times$ C	32	21.4 $\pm$ 0.2	132.5 $\pm$ 4.1	1.34 $\pm$ 0.01
	Q $\times$ Q	22	22.3 $\pm$ 0.5	165.4 $\pm$ 8.6	1.44 $\pm$ 0.02
	Mean		20.9	127.9	1.35
Long day	C $\times$ C	28	20.6 $\pm$ 0.2	114.3 $\pm$ 3.5	1.29 $\pm$ 0.01
	C $\times$ Q	32	22.3 $\pm$ 0.3	155.7 $\pm$ 6.6	1.38 $\pm$ 0.01
	Q $\times$ C	35	22.0 $\pm$ 0.3	148.8 $\pm$ 6.2	1.38 $\pm$ 0.02
	Q $\times$ Q	2	24.3 $\pm$ 0.1	204.0 $\pm$ 9.4	1.42 $\pm$ 0.05
	Mean		21.7	142.3	1.35

June (Table 5). As in experiment 1, the Q $\times$ Q cross showed poor seawater adaptability after long-day treatment whereas the C $\times$ C, C $\times$ Q, and Q $\times$ C groups had developed a high degree of seawater adaptability typical of smolts. As a result, ANOVA for plasma sodium data from all four seawater challenge tests indicated a significant interaction between photoperiod and cross type ($F_{3,421} = 20.8$, $p < 0.001$). One-way ANOVAs for cross type run independently for each photoperiod for combined results from the May and June tests revealed no effect of cross type on seawater adaptability in response to short-day photoperiod ($F_{3,125} = 1.01$, $p > 0.25$). In contrast, there was a significant effect of cross type on seawater adaptability during May and June after long-day treatment ($F_{3,129} = 28.68$, $p < 0.001$). This effect of cross type under long-day photoperiod was still highly significant ($F_{3,128} = 27.5$, $p < 0.001$) after analysis using fork length as a covariate, indicating that it was not due primarily to size differences.

Discussion

In both experiments, Quesnel chinook salmon fry sustained a rapid growth rate and developed a high degree of seawater adaptability only when exposed first to a short-day photoperiod and then to a long-day photoperiod. Clark and Shelbourn (1986) hypothesized that coho salmon (*Oncorhynchus kisutch*) fry

require a period of exposure to short-day photoperiod from the time of first feeding in order to develop uniformly as zero-age smolts. This requirement for exposure to a short-day photoperiod for smolting has since been confirmed in studies with coho and stream-type chinook salmon fry (Clarke et al. 1989; Thorarensen and Clarke 1989; Thorarensen et al. 1989) as well as in the current study. This developmental switch in stream-type chinook salmon responds specifically to daylength experience following emergence; it determines whether they will develop as smolts or remain as parr during the following summer. Under natural conditions where emergence occurs under a long-day photoperiod, it causes juveniles to overwinter in fresh water for at least 1 yr before becoming smolts. However, ocean-type chinook salmon do not share this requirement as shown by the growth and development of the Conuma River fry under either photoperiod regime. The response of the Conuma River fry is consistent with that observed previously in experiments using ocean-type chinook salmon from the Big Qualicum River (Clarke et al. 1989) and with the smolting of these populations as underyearlings under natural rearing conditions (Healey 1991; Taylor 1990a).

Although differences in flesh colour among chinook salmon from the Quesnel River have a genetic basis (Withler 1986), the results of experiment 1 indicate that red- and white-fleshed chinook salmon from the stream-type Quesnel River population have the same response to photoperiod. In contrast, the results

TABLE 5. Plasma sodium concentration ($\text{mmol}\cdot\text{L}^{-1}$) after a 24-h seawater challenge of crosses among Conuma (ocean-type) and Quesnel River (stream-type) chinook salmon under two photoperiods (replicate tanks combined). Values are given as mean $\pm$ SE. $n = 10$ in each test except for June ($n = 24$).

Photoperiod	Cross type ($\sigma \times \varphi$)	Test date				Cross mean
		March 17	April 15	May 18	June 20	
Long day	C $\times$ C	167.1 $\pm$ 1.8	178.7 $\pm$ 5.0	166.1 $\pm$ 2.2	158.8 $\pm$ 0.8	165.1
	C $\times$ Q	181.2 $\pm$ 2.8	177.6 $\pm$ 5.2	161.5 $\pm$ 2.8	165.1 $\pm$ 1.4	169.5
	Q $\times$ C	179.1 $\pm$ 2.9	180.6 $\pm$ 4.6	158.3 $\pm$ 0.9	160.5 $\pm$ 1.2	167.3
	Q $\times$ Q	194.1 $\pm$ 2.9	202.5 $\pm$ 3.3	188.7 $\pm$ 2.7	199.8 $\pm$ 2.7	197.2
	Mean	180.4	185.2	168.6	171.1	174.9
Short day	C $\times$ C	178.9 $\pm$ 2.4	188.5 $\pm$ 4.7	163.4 $\pm$ 1.8	157.1 $\pm$ 0.5	167.9
	C $\times$ Q	183.0 $\pm$ 2.6	195.7 $\pm$ 3.5	160.2 $\pm$ 1.9	161.0 $\pm$ 0.6	171.4
	Q $\times$ C	185.1 $\pm$ 2.9	192.7 $\pm$ 3.5	161.6 $\pm$ 2.2	157.7 $\pm$ 0.6	170.4
	Q $\times$ Q	194.7 $\pm$ 3.3	212.2 $\pm$ 4.8	163.4 $\pm$ 1.8	169.5 $\pm$ 2.7	181.6
	Mean	185.6	197.3	162.2	161.1	172.8

of experiment 2 demonstrate that the difference in responsiveness to photoperiod between juvenile chinook salmon with stream- and ocean-type life history patterns is under genetic control. Fry from both reciprocal Quesnel-Conuma hybrid groups displayed the ocean-type pattern of development. This indicates that the photoperiod-independent phenotype is dominant and not under maternal control. The dependence of growth and smolting in stream-type chinook salmon on photoperiod, and the absence of a photoperiod effect on developmental rate in ocean-type chinook salmon, illustrates a genotype-environment interaction. To our knowledge, this is the first time that genetic control of qualitatively different patterns of growth and smolting has been shown for any salmonid. Hanke et al. (1989) reported a genotype $\times$ environment interaction in presmolt Atlantic salmon (*Salmo salar*) that resulted from a change in the magnitude of the growth response of individual families to a long-day photoperiod during autumn. Within photoperiod treatments, reciprocal hybrid groups were intermediate in their length and weight compared with the pure strains, as would be expected from additive genetic inheritance of growth rate.

In a review of the geographic distribution of these two juvenile life history types in chinook salmon, Taylor (1990a) suggested that variation in age at smolting among populations is controlled in part by environmental modulation (Smith-Gill 1983) of smolting rate through variability in growth opportunity and that this control may be limited by selection for size at migration. Although we agree that variation within each life history type is influenced considerably by differences in growth opportunity, it does not cause a change from one life history type to the other. The present study has demonstrated the existence of a developmental switch, present in stream-type but lacking in ocean-type chinook salmon, that responds specifically to daylength experience in the months after emergence. Patterns of growth and seawater adaptability of stream-type chinook salmon during the subsequent summer are determined by the developmental path taken rather than by growth opportunity. Therefore, we conclude that the difference in juvenile life histories between ocean-type and stream-type chinook salmon results from developmental conversion (Smith-Gill 1983) rather than phenotypic modulation.

In addition to differences in smolting patterns, earlier investigations have reported variation in morphology (Carl and Healey 1984) as well as rheotactic and agonistic behaviours (Taylor and Larkin 1986) between stream-type and ocean-type

chinook salmon fry. However, the shift from juvenile to adult haemoglobin patterns did not vary between the two life history types, nor was it correlated with smoltification (Fyhn et al. 1991). In an earlier study of six populations of chinook salmon, we did not observe a significant difference in weight between fry from ocean- and stream-type populations given early exposure to a long-day photoperiod, nor significant additive genetic variation for weight within populations, after 100 d of rearing in fresh water (Withler et al. 1987). In retrospect, it would seem that the 7–8°C water temperatures used in that experiment were too low to allow expression of the greater growth potential of ocean-type chinook salmon fry when reared under ambient photoperiod. In contrast, Cheng et al. (1987) observed a strong additive genetic effect on growth both in fresh and in salt water of progeny from crosses between two populations of ocean-type chinook salmon. In a study of four populations from each life history type reared in the laboratory under simulated natural photoperiod, Taylor (1990b) found that ocean-type chinook salmon grew more rapidly than did stream-type. Riddell and Sorensen (1986) reported that hybrid yearling coho salmon had growth rates in fresh water that were intermediate to those of the purebred parental strains.

Similarly, quantitative genetic variation for smolting has been observed in accelerated zero-age coho salmon (Saxton et al. 1984; R. E. Withler, unpubl. data). Refstie et al. (1977) reported considerable genetic variation in the proportion of 1-yr smolts among 37 stocks of Atlantic salmon; this proportion was highly correlated with average weight, possibly because they defined a smolt as a fish retained by a 10-mm grader in March. Thorpe and Morgan (1978) demonstrated genetic variation in the proportion of potential yearling smolts among four half-sib families of Atlantic salmon.

The complete dominance of the photoperiod-independent phenotype in hybrid chinook salmon juveniles indicates that photoperiod responsiveness may be under Mendelian genetic control. Nevertheless, we cannot exclude the possibility that it is a threshold trait under quantitative control. Segregation of the photoperiod-dependent and -independent phenotypes among F_2 and backcross progeny is being examined in order to resolve this question. Although not previously demonstrated in salmon, genetic control of responsiveness to short-day photoperiods has been reported in the Djungarian hamster (*Phodopus sungorus sungorus*) by Puchalsky and Lynch (1986, 1988); responsive and nonresponsive hamsters also differed in their expression of

circadian rhythmicity when held under a short-day photoperiod. Studies of mutants that affect circadian clock properties in various organisms over the past 50 yr have revealed different modes of genetic control ranging from single to multiple genes (Feldman 1983). When short-period mutants of *Chlamydomonas* were crossed with the wild-type form, a 50:50 distribution of phenotypes was obtained, demonstrating Mendelian inheritance (Mergenhagen 1984). If photoperiod responsiveness in chinook salmon is in fact controlled by a single gene, it is conceivable that stream-type races may have arisen repeatedly by disruptive selection in ocean-type populations (see Healey 1991).

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References

- BLACKBURN, J., AND W. C. CLARKE. 1987. Revised procedure for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. Can. Tech. Rep. Fish. Aquat. Sci. 1515: 35 p.
- CARL, L. M., AND M. C. HEALEY. 1984. Differences in enzyme frequency and body morphology among three juvenile life history types of chinook salmon (*Oncorhynchus tshawytscha*) in the Nanaimo River, British Columbia. Can. J. Fish. Aquat. Sci. 41: 1070-1077.
- CHENG, K. M., I. M. MCCALLUM, R. I. MCKAY AND B. E. MARCH. 1987. A comparison of survival and growth of two strains of chinook salmon (*Oncorhynchus tshawytscha*) and their crosses reared in confinement. Aquaculture 67: 301-311.
- CLARKE, W. C. 1989. Photoperiod control of smolting: a review. Physiol. Ecol. Jpn. Spec. Vol. 1: 497-502.
- CLARKE, W. C., AND J. E. SHELBOURN. 1986. Delayed photoperiod produces more uniform growth and greater seawater adaptability in underyearling coho salmon (*Oncorhynchus kisutch*). Aquaculture 56: 287-299.
- CLARKE, W. C., J. E. SHELBOURN, T. OGASAWARA, AND T. HIRANO. 1989. Effects of initial daylength on growth, seawater adaptability and plasma growth hormone levels in coho, chinook and chum salmon. Aquaculture 82: 51-62.
- FELDMAN, J. F. 1983. Genetics of circadian clocks. BioScience 33: 426-431.
- FYHN, U. E. H., W. C. CLARKE, AND R. E. WITHLER. 1991. Hemoglobins in smoltifying chinook salmon, *Oncorhynchus tshawytscha*, subjected to photoperiod control. Aquaculture 95: 359-372.
- HANKE, A. R., G. W. FRIARS, R. L. SAUNDERS, AND J. M. TERHUNE. 1989. Family $\times$ photoperiod interaction on growth in juvenile Atlantic salmon, *Salmo salar*. Genome 32: 1105-1112.
- HEALEY, M. C. 1983. Coastwide distribution and ocean migration patterns of stream- and ocean-type chinook salmon, *Oncorhynchus tshawytscha*. Can. Field-Nat. 97: 427-433.
1991. Life history of chinook salmon, p. 311-393. In C. Groot and L. Margolis [ed.] Pacific salmon life histories. University of British Columbia Press, Vancouver, B.C.
- HOAR, W. S. 1988. The physiology of smolting salmonids, p. 275-343. In W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. XIB. Academic Press, New York, NY.
- MERGENHAGEN, D. 1984. Circadian clock: genetic characterization of a short period mutant of *Chlamydomonas reinhardtii*. Eur. J. Cell Biol. 33: 13-18.
- PUCHALSKY, W., AND G. R. LYNCH. 1986. Evidence for differences in the circadian organization of hamsters exposed to short day photoperiod. J. Comp. Physiol. A 159: 7-11.
1988. Characterization of circadian function in Djungarian hamsters insensitive to short day photoperiod. J. Comp. Physiol. A 162: 309-316.
- REFSTIE, T., T. A. STEINE, AND T. GJEDREM. 1977. Selection experiments with salmon. II. Proportion of Atlantic salmon smoltifying at 1 year of age. Aquaculture 10: 231-242.
- RIDDELL, B. E., AND D. A. SORESENSEN. 1986. Growth rate variation between populations of juvenile coho salmon (*Oncorhynchus kisutch*). Aquaculture 57: 374-375. (Abstr.)
- SAXTON, A. M., W. K. HERSHBERGER, AND R. N. IWAMOTO. 1984. Smoltification in the net-pen culture of coho salmon: quantitative genetic analysis. Trans. Am. Fish. Soc. 113: 339-347.
- SMITH-GILL, S. J. 1983. Developmental plasticity: developmental conversion versus phenotypic modulation. Am. Zool. 23: 47-55.
- TAYLOR, E. B. 1990a. Environmental correlates of life-history variation in juvenile chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). J. Fish Biol. 37: 1-17.
- 1990b. Phenotypic correlates of life-history variation in juvenile chinook salmon, *Oncorhynchus tshawytscha*. J. Anim. Ecol. 59: 455-468.
- TAYLOR, E. B., AND P. A. LARKIN. 1986. Current response and agonistic behavior in newly emerged fry of chinook salmon, *Oncorhynchus tshawytscha*. Can. J. Fish. Aquat. Sci. 43: 565-573.
- THORARENSEN, H., AND W. C. CLARKE. 1989. Smoltification induced by a 'skeleton' photoperiod in underyearling coho salmon (*Oncorhynchus kisutch*). Fish Physiol. Biochem. 6: 11-18.
- THORARENSEN, H., W. C. CLARKE, AND A. P. FARRELL. 1989. Effect of photoperiod and various intensities of night illumination on growth and seawater adaptability of juvenile coho salmon (*Oncorhynchus kisutch*). Aquaculture 82: 39-49.
- THORPE, J. E., AND R. I. G. MORGAN. 1978. Parental influence on growth rate, smolting rate and survival in hatchery reared juvenile Atlantic salmon, *Salmo salar*. J. Fish Biol. 13: 549-556.
- WITHLER, R. E. 1986. Genetic variation in carotenoid pigment deposition in the red-fleshed and white-fleshed chinook salmon (*Oncorhynchus tshawytscha*) of Quesnel River, British Columbia. Can. J. Genet. Cytol. 28: 587-594.
- WITHLER, R. E., W. C. CLARKE, B. E. RIDDELL AND H. KREIBERG. 1987. Genetic variation in freshwater survival and growth of chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 64: 85-96.

Deepwater Migrations of Chum Salmon (*Oncorhynchus keta*) along the Pacific Coast of Northern Japan

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Mature chum salmon (*Oncorhynchus keta*) were caught in bottom trawls off Iwate Prefecture, northern Japan, in autumn. To document the incidence and depth distribution of these catches, records were kept of salmon caught by five trawlers that fished along the Pacific coast of northern Honshu during September–December 1986. During this period, 4337 chum salmon were caught at bottom depths ranging from 150 to 460 m with most taken from 200 to 350 m. Gonads and stomach contents were examined for 100 of these salmon. All were mature and close to spawning. Thirty-nine of the 100 stomachs examined were empty and the remaining 61 contained only a small quantity of food, averaging 2.4 g. Chum salmon may move at these depths to avoid the high temperatures of surface waters (12–20°C) found in this area and to follow temperatures close to their thermal preferendum (3–11°C) which are found near bottom. This phenomenon appears to be an adaptation of chum salmon near the southern limit of their range.

Des saumons kétas (*Oncorhynchus keta*) matures ont été capturés à l'automne dans des chaluts de fond au large de la préfecture d'Iwate, dans la région septentrionale du Japon. Afin de documenter la fréquence et la distribution selon la profondeur de ces prises, on a consigné les saumons capturés par cinq chalutiers pêchant sur la côte Pacifique du nord d'Honshu entre septembre et décembre 1986. Durant cette période, 4 337 saumons kétas ont été capturés à des profondeurs de fond variant de 150 à 460 m, la plupart ayant été capturés entre 200 et 350 m. On a examiné le contenu des gonades et de l'estomac de 100 de ces saumons. Il s'agissait dans tous les cas de poissons matures en âge de frayer. Trente-neuf des 100 estomacs examinés étaient vides et les 61 autres ne contenaient qu'une petite quantité d'aliments, soit en moyenne 2,4 g. Le saumon kéta peut se déplacer jusqu'à ces profondeurs pour éviter la température élevée des eaux de surface (12 à 20 °C) qui règne dans cette région et pour retrouver les températures proches de ses préférences thermiques (3 à 11 °C), près du fond. Ce phénomène semble être une adaptation du saumon kéta près de la limite méridionale de son aire de répartition géographique.

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Pacific salmon (*Oncorhynchus*) are the dominant pelagic and anadromous fishes in the North Pacific Ocean and its marginal seas (Moyle and Cech 1982). They are commonly believed to inhabit surface waters throughout their ocean life (Manzer 1964; Manzer et al. 1965; Machidori 1967; Godfrey et al. 1975). Recently, horizontal and vertical movements of salmonids were examined using radio and ultrasonic telemetry (Ishida et al. 1988; Quinn et al. 1989; Ruggerone et al. 1990; Ogura and Ishida 1992). These studies demonstrated that salmonids occurred most frequently near the surface. However, other telemetry experiments showed that chum salmon (*O. keta*) temporarily descended to deep water (deeper than 100 m) (Yano et al. 1984; Soeda et al. 1985). Therefore, it is unclear whether or not chum salmon inhabit deep waters.

Bottom trawlers fishing for groundfish frequently catch chum salmon incidentally at depths over 200 m in autumn off the coast of Iwate Prefecture. Iwate Prefecture is located on the Pacific coast of northern Honshu, where many salmon spawning rivers are located (Fig. 1). Chum salmon are also often caught by trawlers near the bottom at depths between 50 and 250 m off Chiba Prefecture, which is situated about 450 km south of Iwate Prefecture (Fisheries Department of Chiba Prefecture 1988). This information suggests that chum salmon may not always inhabit surface waters.

The probable preferred sea-surface temperature range for chum salmon is 2 or 3 to 11°C and the probable tolerable sea-

surface temperature range is 1–15°C (Manzer et al. 1965). Catch data suggest that the southern limit of chum salmon roughly parallels the 12–13°C surface temperature isotherms (Bakkala 1970). Honshu is near the southern limit of distribution of chum salmon (Neave et al. 1976). Accordingly, chum salmon are thought to frequently encounter warm surface water as a physical obstacle in this area.

This paper describes the distribution, life-history stage, and diet of the salmon caught by bottom trawlers in coastal waters off Iwate Prefecture and relates this information to the physical oceanographic features of the area.

Materials and Methods

To clarify the deepwater distribution of chum salmon, I requested vessel captains to record the location, day, time, trawling depth, and number of chum salmon caught during their trawl operations during the autumn (from September to December) of 1986. (Other species of Pacific salmon are rarely caught in this region of Japan.) The captains of five bottom trawlers agreed to do this, with the exception that the location and day of trawl operations in which no chum salmon were caught would not be reported. Two trawlers used a pair trawl system; the other three trawlers used a single trawl system (Table 1).

Fisheries regulations prohibit trawl fishing within 9 km of the coastline (depths shallower than about 150 m) of Iwate Pre-

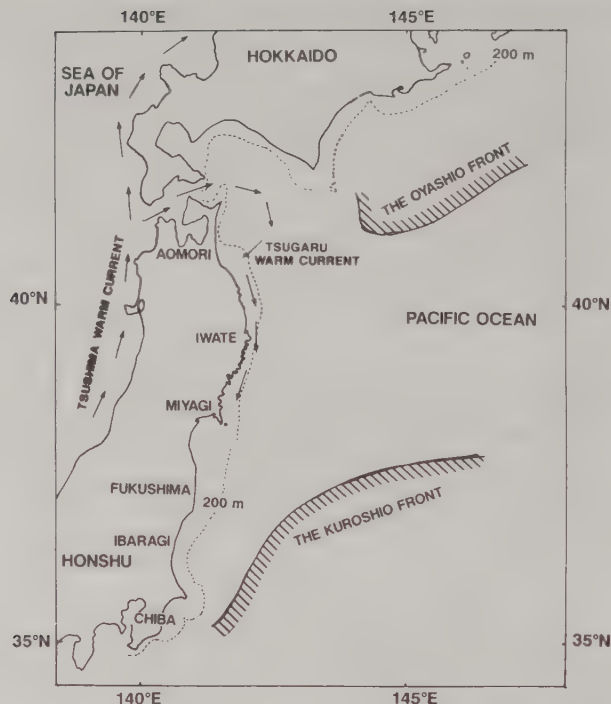


FIG. 1. Survey area showing major water circulation features (from Kawai 1972).

fecture. A trip for trawlers generally lasts 2 or 3 d and the catch comprises mainly codfish, squid, and other demersal species. Each trawling operation usually takes 2–3 h and fishing usually occurs all day and night.

The studies on the life-history stage and stomach contents were done 3 yr after the main study was completed. One hundred chum salmon caught by an additional trawler (60GRT, pair trawl system) in November 1989 were used to investigate the life-history stage and stomach contents of the trawl-caught salmon. The depths of the sampling locations were 260 and 280 m (Table 2). These fish were packed in ice and stored. Within 3 d of capture, the age, sex, gonad weight, fork length, and body weight were determined in the laboratory. Stomach contents were preserved in formalin until identification. Prey

items were sorted into the following major food groups and weighed: Decapoda (Macrura, Euphausiacea), Cephalopoda, fish, and unidentifiable remains.

To determine the characteristics of the environment in which chum salmon were caught, I investigated the vertical water temperature profile in this area, using monthly oceanographic observations made by the Iwate Prefectural Fisheries Experimental Station (Iwate Prefectural Fisheries Experimental Station 1988).

Results

A total of 4337 chum salmon were caught by the five participating trawlers between September and December 1986. Catches gradually increased from October to November and then decreased in December (Table 1; Fig. 2). Catches of chum salmon occurred near the 200-m depth contour and were highest along the northern coast of Iwate Prefecture. There was little change in the area where chum salmon were caught during the 4-mo fishing period.

Pair trawlers caught chum salmon at depths between 200 and 460 m, with highest catch rates occurring at depths of 250–350 m. Single trawlers took chum salmon at depths between 150 and 350 m, with the highest catches at depths ranging from 200 to 300 m (Fig. 3). There was little change in the depth distribution of the catch during the fishing period.

The largest catches of chum salmon occurred during the day; catches at daybreak and during the evening were relatively small. Catches were low at dawn and in the evening, apparently because salmon left the seafloor (Fig. 4). There was little change in this daily pattern during the fishing period.

Biological features of the 100 chum salmon caught by the pair trawler are shown in Table 2. Gonad weights indicated that the chum salmon were mature and, based on the gonad weight criteria of Ishida et al. (1961), near spawning. Of the 100 chum salmon stomachs examined, 61 contained food with an average weight of 2.24 g. No food organisms were found in the stomachs of the other 39 fish (Table 3). Euphausiacea occurred in 72.1% of those stomachs containing food.

Discussion

I personally observed the catch aboard one of the trawlers and found that chum salmon were well mixed in the catches of benthic fishes. Mud from the seafloor was observed in the

TABLE 1. Numbers of salmon caught off the Pacific coast of northern Honshu by vessel and by month, 1986. The number of operations in which salmon were caught is given in parentheses.

Vessel designation	Gross tonnage	Trawl system	September	October	November	December	Total
A	59	Pair trawl	106 (16)	533 (58)	805 (48)	183 (29)	1627
B	59	Pair trawl	77 (13)	674 (49)	977 (62)	200 (40)	1928
C	59	Single trawl	31 (19)	68 (30)	106 (34)	42 (17)	247
D	59	Single trawl	41 (23)	37 (21)	63 (34)	19 (12)	160
E	58	Single trawl	3 (3)	41 (19)	302 (48)	29 (20)	375
Total	—	—	258	1353	2253	473	4337
Percent by month	—	—	5.9 (12.9)	31.2 (29.7)	51.9 (38.0)	10.9 (19.8)	100.0 (100.0)

TABLE 2. Mean fork length, body weight, gonad weight, and age of chum salmon caught by pair trawl in the coastal waters of northern Iwate Prefecture in November 1989. Standard deviations are given in parentheses. Age is the mean in decimal years.

Date	Location	Depth (m)	Sex	Sample size	Fork length (cm)	Body weight (g)	Gonad weight (g)	Age (yr)
Nov. 10	40°07'N, 142°13'E	260	Female	24	66.7 (4.8)	3567 (972)	546 (158)	4.3 (0.6)
			Male	41	65.3 (5.2)	3287 (1003)	173 (54)	4.2 (0.7)
Nov. 11	40°07'N, 142°14'E	280	Female	14	67.1 (5.7)	3467 (960)	520 (175)	4.1 (1.0)
			Male	21	66.7 (5.2)	3454 (913)	181 (59)	3.9 (0.6)
Total	—	—	Female	38	66.8 (5.2)	3530 (969)	536 (165)	4.2 (0.8)
			Male	62	65.8 (5.2)	3344 (977)	176 (56)	4.1 (0.7)

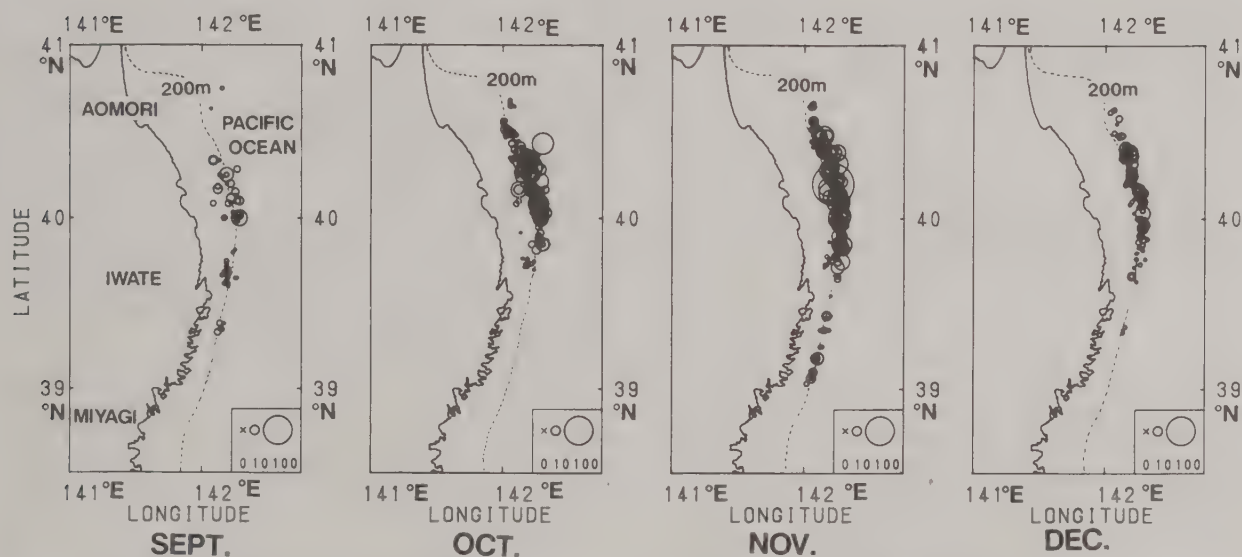


FIG. 2. Monthly distribution of chum salmon catches by bottom trawlers off Iwate Prefecture, 1986. The relative size of circles is proportional to catch.

TABLE 3. Stomach contents of chum salmon caught by pair trawl in the coastal waters of northern Iwate Prefecture in November 1989. Figures in parentheses are based on stomachs containing food.

Prey species	Number of fish	Frequency of occurrence based on stomachs containing food (%)	Total weight of food in sample		Average weight of food in stomachs of fish containing food (g)
			g	%	
Empty	39	—	—	—	—
Containing food	61	100.0	—	—	—
Decapoda					
Macrura	1	1.6	0.94	0.6	0.02
Euphausiacea	44	72.1	50.96	34.7	0.84
Cephalopoda	1	1.6	0.71	0.5	0.01
Fish	7	11.5	51.80	35.2	0.85
Unidentified	21	34.4	42.66	29.0	0.70
Total	100.0	—	147.07	100.0	2.42

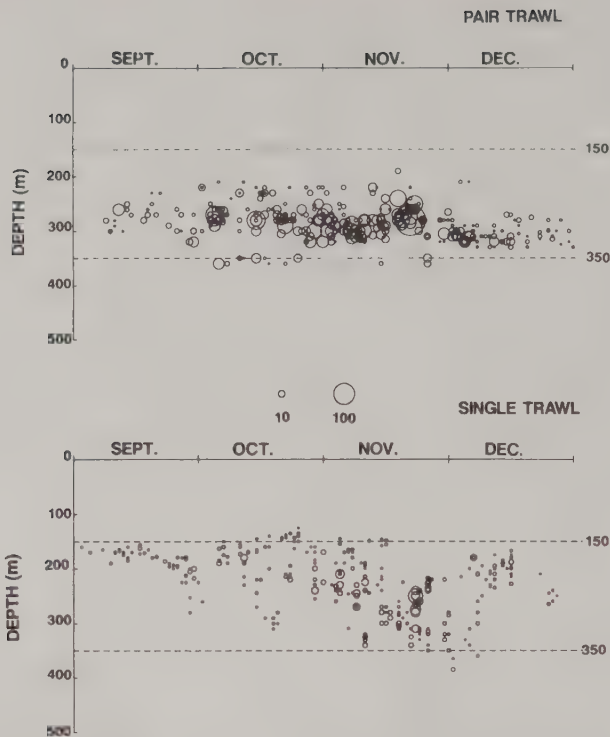


FIG. 3. Relationship between depth and data of chum salmon catches by bottom trawlers off Iwate Prefecture. The relative size of circles is proportional to catch.

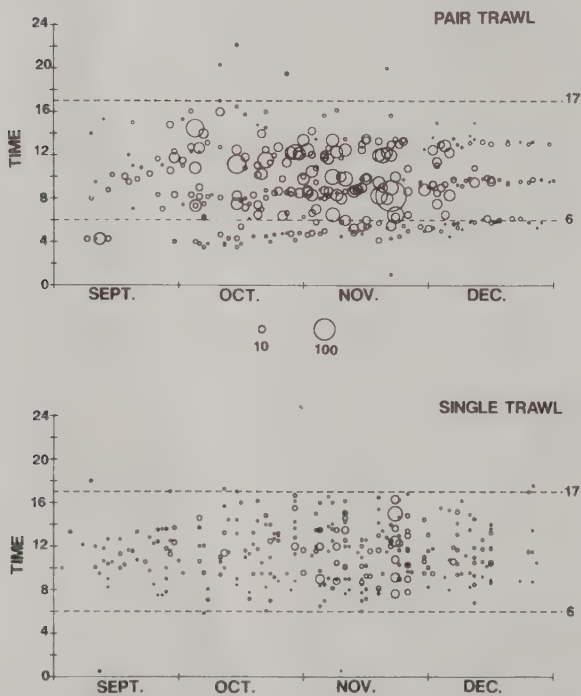


FIG. 4. Relationship between time and date of chum salmon catches by bottom trawlers off Iwate Prefecture. The relative size of circles is proportional to catch.

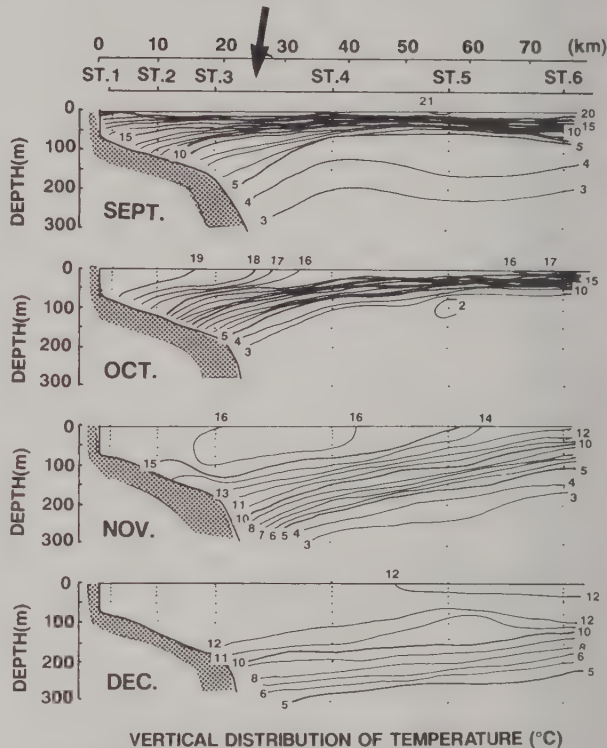
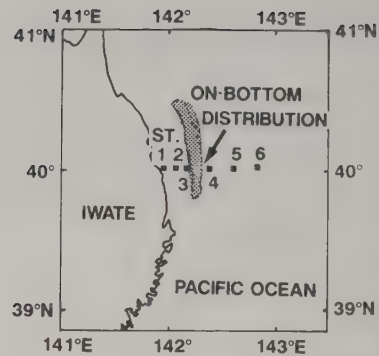


FIG. 5. Deepwater distribution of chum salmon and vertical water temperature profile along 40°N, September–December 1986.

mouth and gill rakers of the chum salmon. The trawler captains stated that most fish caught by trawlers were benthic species with the exception of salmon and that nonbenthic species were rarely taken. Chum salmon taken by the trawlers were therefore likely to have been caught near the sea bottom rather than while the trawl was being raised or lowered.

The depth distribution of the catch (Fig. 3) indicates that chum salmon migrate near the bottom at depths of 200–350 m. In these sea areas, relatively small catches of chum salmon are made by surface gill nets or floating longlines (Ueno et al. 1986).

Recent studies using biotelemetry techniques have revealed that salmonids occasionally descend to a depth of more than 100 m (Yano et al. 1984; Soeda et al. 1985). Mature sockeye salmon (*O. nerka*), for example, have been reported to descend to depths (10–30 m) under the thermocline in waters between Vancouver Island and the mainland of British Columbia to avoid warm surface layers (Quinn et al. 1989).

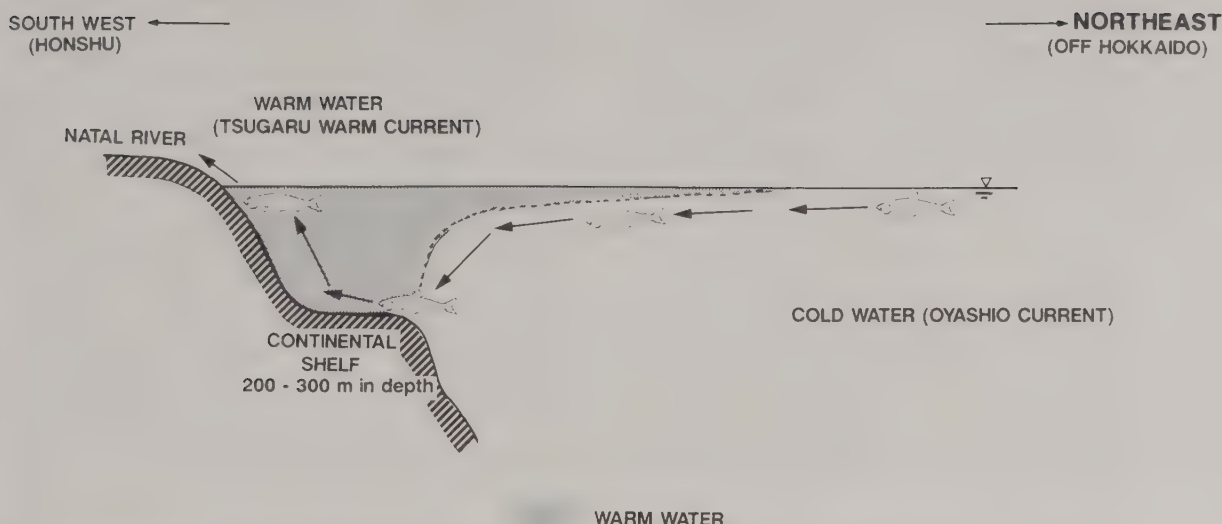


FIG. 6. Schematic diagram of the migratory behavior of chum salmon off the northeastern Pacific coast of Honshu Island based on catches by bottom trawlers.

The monthly vertical section of water properties along 40°N, during September–December 1986, indicated that warm waters (12–20°C) are present in regions shallower than 200 m and above the thermocline, while cold waters (11–2°C) are present in regions deeper than 200 m and below the thermocline (Fig. 5). Generally, the Tsugaru Warm Current (12–21°C) is found within the coastal belt (within 19 km of the coast) of Iwate Prefecture in regions shallower than about 200 m (Ueno and Yamazaki 1987) (see Fig. 1). Water temperatures in the upper layers (0–100 m) of more offshore waters (20–90 km from the coast) range from 12 to 20°C. However, water temperatures at depths of 150–300 m range from 3 to 11°C (Ueno and Yamazaki 1987). The latter temperatures correspond well with the “preferred range” for chum salmon which is from 2 or 3 to 11°C (Manzer et al. 1965). Accordingly, chum salmon may avoid the warm surface layers (Tsugaru Warm Current) in coastal waters of Iwate Prefecture by moving to depths over 200 m. This seems to be a specific adaptation of chum salmon at the southern extent of their range.

The stage of gonadal development shows that the trawl-caught chum salmon would have entered their natal rivers to spawn within a short time. The proportion of empty stomachs is 39% in this study, while Lebrasseur (1966) reported that the proportion of empty stomachs in the northwestern Pacific is 0–3%. Ito (1964) and Lebrasseur (1966) stated that the average weight of the stomach contents of chum salmon in offshore waters of the North Pacific is 4–15 g; however, the value in this study is 2.4 g. The high percentage of empty stomachs and the small amount of food in the stomachs, suggesting low feeding activity, also indicate that the salmon were near spawning. Accordingly, chum salmon are considered to move to nearshore areas and ascend to the surface layers to find their natal river using their olfactory sense (Hasler and Scholz 1983), as illustrated in Fig. 6. When chum salmon returning to the rivers in Iwate Prefecture move to the surface, they must enter warm water (>12°C). Therefore, chum salmon may only move into surface warm water when gonad development requires that they seek their natal rivers.

Acknowledgements

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References

- BAKKALA, R. G. 1970. Synopsis of biological data on the chum salmon, *Oncorhynchus keta* (Walbaum) 1792. FAO Species Synop. No. 41. United States Department of the Interior. Washington, DC. 89 p.
- FISHERIES DEPARTMENT OF CHIBA PREFECTURE. 1988. Showa 62 nendo sake-masu zoshoku sinkoh jigyo chosa hokokusho. (Report on the project to promote salmon propagation in 1987 (Catch distribution of chum salmon by bottom trawlers in coastal waters of Chiba and Ibaraki prefectures.)) Fisheries Department of Chiba Prefectural Government, 260-91, 1-1, Ichiba-cho, Chiba-shi, Japan. 25 p. (In Japanese)
- GODFREY, H., K. A. HENRY, AND S. MACHIDORI. 1975. Distribution and abundance of coho salmon in offshore waters of the North Pacific Ocean. Int. North Pac. Fish. Comm. Bull. 31: 1–80.
- HASLER, A. D., AND A. T. SCHOLZ. 1983. Olfactory imprinting and homing in salmon. Zoophysiology 14: 134 p.
- ISHIDA, K., S. NAGAHORA, K. INOUE, AND T. WATANABE. 1988. Sanriku engan ni kaiki suru sake-singyo no kohdo. (Behavior of adult chum salmon *Oncorhynchus keta* homing to the coast of Sanriku.) Nippon Suisan Gakkaishi 54(8): 1279–1287. (In Japanese with English summary)
- ISHIDA, R., K. TAKAGI, AND S. ARITA. 1961. Criteria for the differentiation of mature and immature forms of chum and sockeye salmon in the Northern Seas. Int. North Pac. Fish. Comm. Bull. 5: 27–48.
- ITO, J. 1964. Kaiyo seikatsu-ki ni okeru sake-masu rui no jiryo to setsuji tokusei ni tsuite. (Food and feeding habit of Pacific salmon (genus *Oncorhynchus*) in their oceanic life.) Bull. Hokkaido Reg. Fish. Res. Lab. 29: 85–97. (In Japanese with English summary)
- IWATE PREFECTURAL FISHERIES EXPERIMENTAL STATION. 1988. Kaiyo-kansoku shiryō, showa 51-62 nen. (Data on monthly oceanographic observation, 1976–1987.) Iwate Prefectural Fisheries Experimental Station, 026, 4-21 Shinhamacho 1-chome, Kamaishi-shi, Iwate, Japan. 402 p. (In Japanese)
- KAWAI, H. 1972. Kuroshio to Oyashio no kaikyo-gaku. (Oceanography of Kuroshio and Oyashio.), p. 129–321. In Editorial Committee for Basic Oceanography [ed.] Basic oceanography. 2. Tokai University Press, Tokyo, Japan. (In Japanese)
- LEBRASSEUR, R. J. 1966. Stomach contents of salmon and steelhead trout in the northeastern Pacific Ocean. J. Fish. Res. Board Can. 23: 85–100.

- MACHIDORI, S. 1967. Hokusei taiheiyō ni okeru sake-masu rui no suichoku bunpu II. (Vertical distribution of salmon (genus *Oncorhynchus*) on the north-western Pacific — II.) Bull. Hokkaido Reg. Fish. Res. Lab. 32: 13–20. (In Japanese with English summary)
- MANZER, J. I. 1964. Preliminary observations on the vertical distribution of Pacific salmon (genus *Oncorhynchus*) in the Gulf of Alaska. J. Fish. Res. Board Can. 21: 891–903.
- MANZER, J. I., T. ISHIDA, A. E. PETERSON, AND M. G. HANAVAN. 1965. Salmon of the North Pacific Ocean. Part V. Int. North Pac. Fish. Comm. Bull. 15: 449 p.
- MOYLE, P. B., AND J. J. CECHE, JR. 1982. Fishes: an introduction of ichthyology. Prentice-Hall, Inc., Englewood Cliffs, NJ. 593 p.
- NEAVE, F., T. YONEMORI, AND R. G. BAKKALA. 1976. Distribution and origin of chum salmon in offshore waters of the North Pacific Ocean. Int. North Pac. Fish. Comm. Bull. 35: 1–79.
- OGURA, M., AND Y. ISHIDA. 1992. Swimming behavior of coho salmon, *Oncorhynchus kisutch*, in the open sea as determined by ultrasonic telemetry. Can. J. Fish. Aquat. Sci. 49: 453–457.
- QUINN, T. P., B. A. TERHART, AND C. GROOT. 1989. Migratory orientation and vertical movements of homing adult sockeye salmon, *Oncorhynchus nerka*, in coastal waters. Anim. Behav. 37: 587–599.
- RUGGERONE, G. T., T. P. QUINN, I. A. MCGREGOR, AND T. D. WILKINSON. 1990. Horizontal and vertical movements of adult steelhead trout, *Oncorhynchus mykiss*, in the Dean and Fisher channels, British Columbia. Can. J. Fish. Aquat. Sci. 47: 1963–1969.
- SOEDA, H., K. YOZA, T. SHIMAMURA, E. HASEGAWA, AND K. YOSHIWARA. 1985. Siretoko-hanto engan-iki ni okeru souki raiyu Sirozake no suichokuido. (On the vertical moving behavior of chum salmon early migratory seasons off the coast of Shiretoko Peninsula.) Nippon Suisan Gakkaishi 51(9): 1425–1429. (In Japanese with English summary)
- UENO, Y., K. NAGAHORA, K. OIKAWA, N. YAMAZAKI, AND M. YAMANE. 1986. Iwate-ken engan-iki ni okeru aki-sake no bunpu, kaiyu, seibutsugaku-teki tokusei oyobi haenawa gyogyō (Aki-sake gyogyō chōsa hokoku). (Migration, distribution, biological characteristics, and longline fishing gear of autumn chum salmon in 1984 (Report on the experimental fisheries of autumn chum salmon in 1984.)) Iwate Prefectural Fisheries Experimental Station, 026, 4-21 Shinhama-cho 1-chome, Kamaishi-shi, Iwate, Japan. 46 p. (In Japanese)
- UENO, Y., AND M. YAMAZAKI. 1987. Sanriku engan-iki ni okeru Tsugaru Danryū no kisetu henka. (Some features of seasonal changes in the Tsugaru Warm Current along the Sanriku coast.) Bull. Tohoku Reg. Fish. Res. Lab. 49: 111–123. (In Japanese with English summary)
- YANO, K., T. ICHIHARA, A. NAKAMURA, AND S. TANAKA. 1984. Escape behavior of the chum salmon *Oncorhynchus keta* upon encountering Dall's porpoise *Phocoenoides dalli*. Nippon Suisan Gakkaishi 50(8): 1273–1277. (In English with Japanese summary)

Planning Models for Individual Transferable Quota Programs¹

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This paper develops an approach to simulating markets for individual transferable quotas prior to their actual implementation. This approach is based on linear programming models for individual vessels that allow estimation of market derived demand for quota to simulate the expected equilibrium market price for quota and maximum quasi-rents for alternative quota allocations. The simulated price is an annual lease or rental price. The approach, applied to a sample of longline vessels targeting sablefish (*Anoplopoma fimbria*) in the Columbia INPFC area of the United States, indicates substantial potential gain in quasi-rent and economic efficiency from quota trade. The simulated quota exchange also indicates potential for concentration of quota. The quota market risks becoming thin, noisy, and hampered by noncompetitive forces, potentially requiring limits to quota transfer and concentration.

Le présent article élabore une approche pour la simulation des marchés des quotas individuels transférables avant leur mise en oeuvre réelle. L'approche est basée sur des modèles de programmation linéaire pour les navires individuels qui permettent d'estimer la demande du marché en quotas afin de simuler le prix d'équilibre du marché prévu pour le quota et les quasi-rentes maximales pour les autres allocations de quotas possibles. Le prix simulé est un prix de crédit-bail ou de location annuel. L'approche, appliquée à un échantillon de palangriers pêchant la morue charbonnière (*Anoplopoma fimbria*) dans le secteur INPFC Columbia aux États-Unis, indique un gain potentiel substantiel en matière de quasi-rentes et d'efficacité économique découlant du commerce des quotas. La simulation des échanges de quotas indique également un potentiel de concentration des quotas. Le marché des quotas risque de devenir faible, tumultueux et entravé par des forces non concurrentielles, ce qui pourrait nécessiter l'imposition de limites au transfert et à la concentration des quotas.

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1. Introduction

Individual transferable quotas (ITQs) are tradable property rights over the flow of the resource stock (Scott 1986). Each vessel is allocated a quantity of the total harvest for which it has a right to catch over some time period. ITQs are now earning the attention of fishery managers worldwide as a means to regulate common property fishing industries (Muse and Schelle 1989).

Several fundamental issues concern regulators and industry when planning or considering the feasibility of an ITQ program. First is the expected equilibrium price and economic rents (total revenue less the payment required to bring into production an input fixed in supply). A second concern is the gains in economic efficiency from quota trade.³ Third, regulators are concerned about potential quota and industry concentration and competitiveness as well as thinness (number of participants) of the quota market. Resolution of these sources of uncertainty

would enable better evaluation and planning of prospective ITQ programs.

This paper is expository, with several purposes. First, the paper aims to facilitate transfer of information from economics to fisheries management. Second, the paper develops a simulation model of an ITQ market based on linear programming models for individual vessels that can address the above concerns about a proposed ITQ program while it is still in the planning stages. Third, the simulation model estimates economic benefits from a potential ITQ program in a longline fishery for sablefish (*Anoplopoma fimbria*) off Oregon and Washington in the United States and estimates the expected price of quotas for alternative allocations among vessels. The efficiency gains from quota trade and expected equilibrium ITQ price from the simulation model are short-run, since they are conditional upon the existing vessels; Squires et al. (1992) addressed the expected entry/exit of vessels and industry structure of the fleet. The simulated price is an annual lease or rental price.

The paper proceeds as follows. Section two discusses important features of an economic model of ITQs and a survey of the relevant modeling literature, ITQ price formation, background information on the longline fishery for sablefish, a linear programming model to estimate the vessel's short-run production technology and unit quota rents, which form the basis of the simulation, and short-run equilibrium price formation of the ITQ and expected quasi-rents and gains from trade. Section three reports empirical results from the simulation model and discusses policy implications for an ITQ management program.

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³Gains from trade, i.e. arbitrage efficiency, due to production are the increase in value of output (producer surplus) that occurs due to trade between producers. This exchange allows producers to specialize according to their comparative efficiencies. Gains from trade can also include consumption gains (consumer surplus). Only producer gains are considered because output prices are assumed constant in this paper.

2. Materials and Methods

2.1. Features of an Economic Model for ITQs and Literature Review

The economic model of ITQs rests upon the foundation initially developed in the environmental pollution (Dales 1968; Baumol and Oates 1971; Montgomery 1972; Weitzman 1974) and international trade (Anderson 1988) literatures and extended by Christy (1973) and Moloney and Pearce (1979) to fisheries. This framework builds upon the individual firm (vessel), explicitly recognizing that the market for ITQs is formed by individual firms exchanging quota. This paper uses firm and vessel interchangeably but recognizes that some firms may be multivessel.

The fisheries literature on ITQs (Christy 1973; Moloney and Pearce 1979; Copes 1986; Scott 1986; Lindner et al. 1989; Muse and Schelle 1989; Sissenwine and Pace 1992) has not fully recognized two other important points. The first (Lau 1976) recognizes that the vessel's (firm's) production decisions are made in two steps. In the first step, the vessel maximizes profits (total revenue less operating costs) in the short term from the existing capital stock (vessel and equipment). (These short-run profits are also called quasi-rents or producer surplus; see Just et al. 1982.) In the second step, the vessel adjusts, over a longer time period, its capital stock (e.g. change vessels) to the appropriate size to maximize long-run profits.

The second point (Schulze and d'Arge 1974) is that both short- and long-run economic efficiency have two requirements when controlling an externality (a cost external to the vessel but which affects other vessels in the fishery, such as the abundance of fish in open-access fisheries). The first requirement is that the marginal value of the vessel's output must equal marginal cost (both internal and external to the vessel). The second requirement is that the total value of the vessel's output must not be less than total costs (both internal and external to the vessel).

Taken together, these points imply that a single, long-run equilibrium ITQ price does not immediately develop. Instead, equilibrium ITQ prices form over short-run periods as individual vessels exchange quota. Over a longer time period, vessels evaluate all revenues and costs, including the expected ITQ price and their implicit marginal valuation of their capital stock, and then adjust their capital stock or technology by changing vessel size, gear, or electronics, or by leaving the industry. New participants may also enter the industry. Although beyond the scope of this paper, it is also possible that changes in industry structure may change fish stock biomass and production costs, which will induce additional changes in the industry.

An ITQ price subsequently forms for the corresponding long-run equilibrium structure of the fleet. This price includes the user cost of the unpriced resource given the existing fleet size and structure and an optimally set quota (Moloney and Pearce 1979). The user cost of the resource is the opportunity cost to the user of taking a unit of the resource in the present period and represents the shadow price of the fish stock (Clark 1976). This long-run price is the present value of the net returns from this asset.

The vessel's investment and entry/exit decision and long-term ITQ price formation can take varied lengths of time. In the Australian bluefin tuna (*Thunnus thynnus*) and U.S. surf clam (*Spisula solidissima*) fisheries, industry restructuring proceeded fairly rapidly, while the process has been protracted

in New Zealand (Geen and Nayer 1989; Lindner et al. 1989; Campbell 1990; Anonymous 1991).

In sum, economic models of ITQs should recognize that ITQ markets are formed by individual vessels (firms) exchanging quota and that different ITQ prices exist for different periods.

The issue of competitive equilibrium market prices must also be addressed by simulation models of ITQ markets. Hahn (1984) and Misiolek and Elder (1989) examined non-competitiveness or monopoly power in the market for tradable property rights over pollution, and Anderson (1991) extended the analysis to fisheries. When simulating the initial, short-run ITQ price for the existing fleet, lack of market competitiveness from a small number of participants may not necessarily pose a problem, since quota is typically allocated amongst an overcapitalized fleet with excessive vessel numbers. Also, evaluating the vessels' investment and entry/exit decisions and likelihood for quota trade sheds light upon this issue.

The paper's simulation generates a single competitive equilibrium price for ITQs, but in practice one may not form. Lindner et al. (1989) and Lindner (1990) found evidence of considerable price dispersion (widely distributed prices rather than a single equilibrium price) and sequential, bilateral exchange in New Zealand. Nonetheless, simulating the expected equilibrium ITQ price for the existing fleet provides the best possible estimate of the expected price and also a basis for comparing alternative policies.

Empirical studies of ITQs in fisheries have only recently emerged. Geen and Nayer (1989) assessed ITQs in a retrospective or ex post framework for the Australian bluefin tuna. Their industry-wide bioeconomic model generated the long-run equilibrium price of quota and industry structure. Geen et al. (1990) applied linear programming to assess a priori a program of individual quotas in the multispecies southeast trawl fishery in Australia. Individual quotas could not be explicitly transferred in the model and the effects of quota trading on adjustments in production were incorporated into the model indirectly. Hence, this model primarily focused upon the long-run effects of investment and industry restructuring under ITQs. Haynes and Pascoe (1988), in a linear programming study of the northern prawn (*Pandalus borealis*) fishery of Australia, noted that the results of an industry-level model correspond to a sole owner (solving the incomplete property rights structure of a common property fishery, thereby alleviating the stock effect externality) and hence give a theoretically identical result to that from a program of ITQs. This approach, while theoretically correct, assumes a common, single (aggregate) production technology for each vessel grouping. Most importantly, this approach does not allow fully analyzing the likely operation of the ITQ market to assess the hypothetical competitive equilibrium market price, resource rents (total revenue less costs), gains in efficiency from trade, and distribution of the licenses and trade patterns.

Squires (1990, 1991), following the two-step procedure above, simulated a potential ITQ program for trawlers. The expected short-run equilibrium ITQ price was estimated and the effect of the ITQ price upon the individual vessel's investment or disinvestment decision assessed. Econometric studies, such as this one, suffer from the possible failure of the model to be well behaved and from its parametric form, i.e. an explicit functional form must be imposed on the data. The econometric approach can handle statistical noise, but it imposes an explicit and possibly overly restrictive functional form for the production technology.

An alternative vessel-level approach is possible, drawing from the study of production efficiency in the economics literature (Lovell and Schmidt 1988). One strand of this literature circumvents the parametric assumptions of the econometric approach by representing each individual firm's production as a separate linear programming problem. Hence, studies of prospective ITQ markets can specify a linear program for each vessel, impose quota and calculate the unit quota rent, use this price (unit rent) – quantity relationship to simulate the ITQ market, and determine the ITQ's expected equilibrium price. This study develops this framework in a prototype procedure, adapting the empirical approaches of Hahn and Noll (1983), Anderson (1988), and Squires (1991) to linear programming to give the expected annual lease or rental price under certainty.

The deterministic qualities of the linear programming approach circumvent many econometric problems, but linear programming has limitations of its own. Linear programming imposes constant returns to scale (a 1% increase in inputs increases output by 1%), fixed proportions among outputs (Leontief technology), and nonjointness-in-inputs (separate production processes for each output or groups of outputs). Moreover, because of the linearity, profitable activities are employed subject solely to resource constraints rather than diminishing returns (e.g. rising operating costs as search time increases) and changing prices. Hence: (1) calibration of the model to observed production is difficult without adding a number of additional, ad hoc constraints and (2) large changes in the solution (basis) are often observed for small changes in the constraints, prices, or technical coefficients.

Other linear programming studies of fisheries include Brown et al. (1979), Siegel et al. (1979), Meuriot and Gates (1983), Overholtz (1985), Huppert and Squires (1987), Haynes and Pascoe (1988), and Geen et al. (1990).

2.2. ITQ Price Formation

After the allocation of quota but prior to its exchange, each unit (e.g. pound, percentage) of the quota has an implicit economic value or unit economic rent. For the profit-maximizing vessel, this unit rent τ , is the vessel's unit quasi-rent (producer surplus). That is, τ is the difference between the marginal revenue from production P (the increase in total revenue for a one-unit increase in output) and the vessel's marginal cost (MC, the increase in cost for a one-unit increase in output), i.e. $\tau = P - MC$. (With joint production, the quota's virtual price replaces marginal cost (Squires and Kirkley 1991). Virtual prices are those prices which would induce an unconstrained firm to behave in the same manner as when faced with a given vector of quantity constraints; see Neary and Roberts (1980) for further discussion.)

For a single year, τ is conditional upon the capital stock (vessel and equipment) and short-run marginal costs and is equivalent to the price of annual lease or rental quota measured as a quasi-rent (Campbell 1990). Over a longer period, when capital (vessel, equipment) has time to adjust, τ becomes the net present value of the expected net earnings stream, i.e. $\tau = \sum_t (P_t - LMC_t)/(1 + \delta)^t$, where δ is the discount rate, $t = 1, \dots, T$, T is the planning horizon, LMC_t is expected long-run marginal cost (marginal cost when all inputs can vary) in time t , and P_t is the expected output price in time t .

This unit quota rent τ varies among firms according to the output and input prices they face and their marginal costs (which are determined by their production technology and prices for inputs such as fuel and labor). The important point here is that

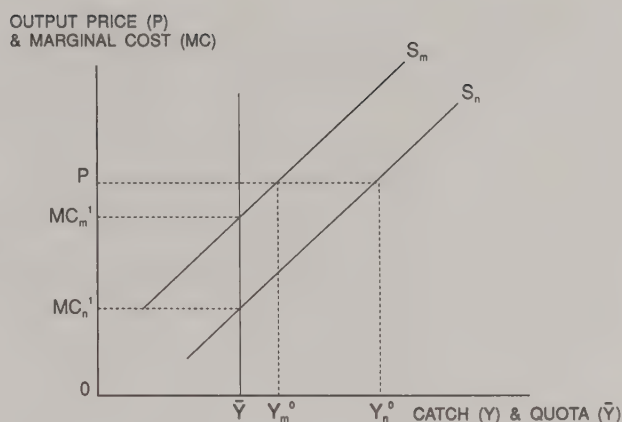


FIG. 1. Quota market for two vessels.

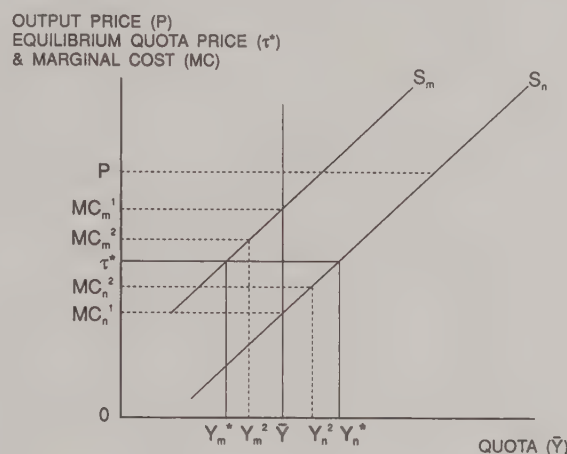


FIG. 2. Quota exchange process.

for exogenous and fixed output prices, the most efficient vessel (with lowest marginal costs) will place a higher value on a unit of quota than an inefficient one, i.e. $\tau_n > \tau_m$ because $MC_n < MC_m$. Consequently, a vessel n will demand quota from (supply to) firm m when $\tau_n > (<) \tau_m$. While the price paid for these species is constant and determined exogenously, MC varies with the level of quota held, so that quota purchase (sale) monotonically lowers (raises) τ in the quota market until an equilibrium market price for quota τ^* is formed and the value of a unit quota is the same for all vessels ($\tau_n = \tau_m$) at the last unit exchanged.

Figure 1 (Squires 1990) illustrates the quota market for two vessels m and n . The supply curve (equal to marginal cost and reflecting diminishing returns in this example) for vessel m lies above that for vessel n , indicating that vessel n is more efficient, i.e. can produce any given output level at lower cost. Prior to quota, both vessels face the same exogenous price for fish P , and vessel n (m) produces Y_n^0 (Y_m^0). Under a quota of $\bar{Y}$, vessel n (m) faces marginal cost MC_n (MC_m). Hence, $\tau_n = P - MC_n > \tau_m = P - MC_m$ and vessel n will buy quota from vessel m .

Figure 2 illustrates how quota exchange progressively narrows τ between vessels, until unit quota rents are equalized at the last unit traded, i.e. $\tau_m = \tau_n$, and the equilibrium ITQ price τ^* is formed. Initially, each vessel faces market price P and catches Y_m^0 and Y_n^0 (not shown). Each vessel is subsequently

allocated $\bar{Y}$ and faces a unit quota rent of $\tau_m = P - MC_m^1$ or $\tau_n = P - MC_n^1$. Vessel m sells quota to vessel n , since $\tau_m < \tau_n$, causing τ_m to rise and τ_n to fall, reaching an intermediate position such as $P - MC_m^2$ and $P - MC_n^2$ with quota holdings Y_m^2 and Y_n^2 . Trade continues until the unit quota rents are equalized for the last unit of quota exchanged, giving final quota holdings of Y_m^* and Y_n^* . In this two-vessel example, vessel n 's final purchase ($\bar{Y} - Y_n^*$) equals that sold by vessel m ($\bar{Y} - Y_m^*$).

The efficient outcome after competitive exchange of quota is given by equalizing the net returns from holding quota, τ , across all N vessels in the industry, i.e. $\tau_1 = \tau_2 = \dots = \tau_N = \tau^*$. In practice, τ^* differs between vessels by an amount equal to costs from transactions, information, transportation, risk, and uncertainty (these amounts vary by vessel).

Quota exchange amongst vessels gives gains from trade due to reduction in production costs (also called arbitrage efficiency). Economic rents from the species are maximized and gains from trade are enjoyed as less efficient vessels trade quota to more efficient vessels. The full economic rent generated by the resource, $\tau^* \bar{Y}$, where $\bar{Y}$ is the overall industry quota for the species, is also the revenue government receives if quota were initially auctioned at a single price or were placed under a uniform tax (Weitzman 1974; Anderson 1988). In theory, at least, the initial distribution of quota only affects the distribution of rents among vessels, not the efficiency after exchange (Montgomery 1972). If $\bar{Y}$ is optimal, then the equilibrium quota price formed after exchange, τ^* , is the marginal user cost of the unit resource (Moloney and Pearse 1979).

When linear programming is used to model the firm's short-run production technology (i.e. capital is fixed), short-run marginal costs (SMC) equal average variable costs (AVC) because of the constant returns to scale (a 1% increase in inputs yields a 1% increase in output) assumed in a linear programming model. Assuming competitive and exogenous prices, $\tau = P - SMC = P - AVC$. The unit quota rent prior to exchange τ , i.e. the firm's implicit marginal valuation of quota, is given with linear programming models by the shadow value of the quota constraint (the increase in profit with a one unit increase in the quota constraint). That is, the linear program measures the value of a unit quota to a vessel by estimating the change in a vessel's profits for an additional unit of quota. If Z denotes the vessel's short-run profits and $\bar{y}$ is the vessel's allocated quota, then $\tau = \delta Z / \delta \bar{y}$, where δ is partial derivative. The gains in net revenue with a one unit increase in $\bar{y}$ include net revenue gains from bycatch as well as from the regulated species because vessels can catch more of other species.

2.3. Sablefish Longliners of Columbia INPFC Area

The ITQ simulation was applied to the Columbia management region, which runs from Cape Blanco, Oregon (43°00'N), to Cape Elizabeth, Washington (47°30'N). The region's ports include Coos Bay, Waldport, Newport, Tillamook, Astoria, and Portland in Oregon and Ilwaco and Westport in Washington. The relatively small number of ports makes the Columbia region an easy area in which to monitor landings, which would be important in an ITQ program.

Fixed gear (longline and fish pot) and groundfish trawl operators in the Pacific sablefish fleet dispute the allocation of allowable sablefish harvest, with fixed gear and trawl vessel groups each desiring a larger share. Strong Japanese sablefish demand for U.S. exports supports high ex-vessel prices of sablefish, further elevating the gear conflict in the sablefish fleet. These factors, the limited number of ports, longevity, and

relative stability of recruitment to the sablefish biomass (which results in stable resource supply), make it a potential species for a market-based solution to the allocation issue.

2.4. Empirical Model

The linear programming model for the Columbia sablefish fishery is a modified version of that employed by Huppert and Squires (1987) to estimate the optimal size of the Pacific coast trawl fleet. The model maximizes the vessel's short-run profit (quasi-rent or producer surplus; Just et al. 1982), conditional upon the vessel or capital stock, subject to constraints on total allowable annual harvest and on vessel-weeks fished and the standard nonnegativity constraints (no variable is negative). The decision variable for each vessel is the number of weeks fished W_j in each quarter j ($j = 1, 2, 3, 4$) of the year, i.e. vessels vary weeks fished to maximize quasi-rent (short-run profits). The model is specified for each vessel as

$$(1) \quad \max Z = \sum_i \sum_j W_j (P_{ij} a_{ij} - VC_s)$$

W_j

subject to

$$(2) \quad \sum_j W_j a_{ij} \leq y_i \quad \text{for all } i,$$

$$(3) \quad W_j \leq 10 \quad \text{for all } j,$$

$$(4) \quad \sum_j W_j \leq \bar{W},$$

and

$$(5) \quad W_j \geq 0 \quad \text{for all } j.$$

Quasi-rent for each vessel, Z , is total revenue less variable costs. The exogenous ex-vessel price for species $i = 1, \dots, 18$ in quarter j is denoted P_{ij} . Variable cost per week for vessel size class s ($s = 1, \dots, 5$), VC_s , is the short-run (operating) costs per week per vessel. The technical coefficients, a_{ij} , are weekly catch rates, i.e. tons of species i harvested per week by the vessel in quarter j . Each vessel's production limit for species i is denoted y_i . If the vessel is subject to quota, then for species i , $\bar{y}_i$ indicates the quota level for the individual vessel.

The first constraint, equation (2), represents the annual limits on each vessel's catch of species i . The second constraint, equation (3), constrains the harvesting activity of a longliner to 10 wk in a quarter. The third constraint, equation (4), is an annual constraint which requires the optimum annual sum of weeks fished by each vessel to not exceed the actual annual weeks fished $\bar{W}$. The actual weeks fished was less than the annual constraint of 40 wk implied by equation (3). The fourth constraint, equation (5), represents the standard nonnegativity constraint for the decision variable, weeks fished (W_j).

The 18 species potentially harvested by vessels are Dover sole (*Microstomus pacificus*), English sole (*Pleuronectes vetulus*), petrale sole (*Eopsetta jordani*), Pacific halibut (*Hippoglossus stenolepis*), other flatfish, lingcod (*Ophiodon elongatus*), Pacific cod (*Gadus macrocephalus*), Pacific hake (*Merluccius productus*), Pacific ocean perch (*Sebastes alutus*), widow rockfish (*Sebastes entomelas*), yellowtail rockfish (*Sebastes flavidus*), thornspine thornyhead (*Sebastolobus alascanus*), longspine thornyhead (*Sebastolobus altivelis*), other rockfish, salmon, albacore, shrimp, crab, and a group of other miscellaneous species. Over the course of the year, most vessels tend to concentrate production upon sablefish and some combination of halibut, lingcod, and rockfish.

The liner programming approach to optimizing the vessel's short-run profit is conditional upon existing vessel size, vintage (age), and state of technology (electronics, design, etc.). The underlying bioeconomic model is one of certainty and short-run static equilibrium. Hence, fish prices and input prices are assumed fixed and exogenously determined, fish stock densities and harvest rates do not depend upon the level of harvest, and the harvesting technology is constant (Huppert and Squires 1987).

These assumptions are reasonable for a vessel-level model of a region, since prices are determined coastwide over all gear types. Moreover, sablefish and thornyhead prices are likely to be exogenously determined in the Tokyo wholesale market (current research is addressing this issue). Dockside fuel prices are set by a standard mark-up procedure following the terminal price in Seattle or San Francisco. Some variation is observed coastwide, reflecting differences in marketing margins and perhaps product form and quality and competitiveness of the market structure.

Finally, the model does not consider the costs of management or optimal level of resource use. Instead, the optimal level of each ITQ-managed species, in this case only sablefish, is exogenously determined. Solving the long-run optimal level would require solution of each firm's long-run optimum level of capital stock, the optimum aggregate capital in the industry, and optimal abundance level.

2.5. Data and Variable Construction

A total of 139 longliners operated in the Pacific coast ground-fish fishery in 1987 (PacFIN Research Data Base), of which 105 landed at least 0.5 metric ton of sablefish in the Columbia area (PacFIN Research Data Base). From these 105 vessels, the top 30 revenue producers or "highliners" were selected to insure individual vessels in the model covered their short-run costs and to simplify the modeling process.

Five size classes of vessels were selected for the study: (1) ≤ 15.0 m, (2) 15.1–16.0 m, (3) 16.1–18.0 m, (4) 18.1–20.0 m, and (5) ≥ 20.1 m. The size composition of the sample fleet, based on U.S. Coast Guard registered length, generally reflected the Pacific longline fleet. The number of vessels in each class was (1) 14, (2) 5, (3) 5, (4) 4, and (5) 2.

Highliners in Columbia landed approximately 20% of the sablefish landed coastwide by longliners during 1987 (Natural Resources Consultants 1988). The Columbia landings include multispecies catch (2519.52 metric tons) valued at \$4.1 million (1987 U.S. dollars), consisting mainly of sablefish (889.87 metric tons) valued at \$1.3 million. The mean ex-vessel price per metric ton for sablefish was \$1606.30, which was higher than the coastwide 1987 ex-vessel price of \$1040.00.

Technical coefficients a_{ij} were computed as weekly average catches (in metric tons) by quarter for each vessel from the 1987 PacFIN Research Data Base. Each "week" of fishing represents one calendar week in which at least one commercial landing was recorded. Week was selected rather than trip, since larger vessels make longer but fewer trips than smaller vessels and the latter may make several trips in a week. The choice of trip tends to otherwise overstate the time fished of smaller vessels.

Quarterly species prices were quarterly ex-vessel arithmetic means from the PacFIN Research Data Base. Variable costs included the costs of fuel, oil, provisions, ice, bait, gear replacement, crew, and bonuses. The labor costs were observed costs rather than estimates of opportunity costs. These cost data were obtained from a random sample survey of coastwide

sablefish longline and fish pot vessels for 1987. Median rather than mean variable costs were computed because of the skewed and relatively limited sample size for each vessel size class.

2.6. Quota Allocation

Six different levels of overall quota $\bar{Y}$ were allocated among the 30 vessels for the simulation. The first overall quota was set at the total metric tons harvested by the sample of Columbia sablefish longliners in 1987. Subsequent industry sablefish quotas in the model were set at 90, 80, 70, 60, and 50% of the initial industry quota. Each vessel was allocated quota in proportion to its share of the actual catch in 1987 as a percentage of the target level, rather than in absolute units. In the model, quota is assumed issued without charge, to be freely transferable, and sufficiently divisible so that the small denominations and large numbers contribute to an active and competitive market in quota. The problem considers a certain and static environment and does not allow banking of quota for future use.

2.7. Derived Demand for Sablefish Quota

Demand curves show quantity demanded of a commodity as a function of its price and other exogenous factors. Inverse demand curves show price as a function of quantity demanded and the other exogenous factors.

Vessels have a derived demand for quota. That is, given ex-vessel fish prices and technology, the vessel's demand curve for quota is derived from final demand for that species and the vessel's costs and production technology (Squires 1990, 1991). To estimate the derived demand for quota used in the simulation model of the ITQ market, the linear programming model is first run to obtain estimates of the unit quota rent τ (which are the shadow prices for different vessel quotas $\bar{y}$). The vessel's inverse derived demand for the sablefish quota is then estimated by regressing τ upon the vessel's quota ($\bar{y}$) and dummy variables (D_s) for different vessel size classes, where the intercept α_0 represents the smallest vessels:

$$(6) \quad \tau = \alpha_0 + \sum_{s=2}^5 \alpha_s D_s + \beta \bar{y}.$$

Ordinary least squares estimates are potentially biased because τ is censored at zero. That is, not all vessels are bound by quota, so that the shadow value derived from the linear programming model can be zero. In such instances, Tobin's censored regression technique is appropriate (Maddala 1985).

2.8. Equilibrium Market Quota Price

The estimated inverse derived demands for individual vessels, from equation (6), were summed over the N (30) vessels to get the inverse market-derived demand curve for an overall quota $\bar{Y}$:

$$(7) \quad \tau^* = \alpha_0 + \frac{1}{N} \sum_{i=1}^N \sum_{s=2}^5 \alpha_s D_i + \frac{1}{N} \beta \bar{Y}.$$

The equilibrium market price for quota in the short run, τ^* , conditional upon the existing fleet capital stock, is the price that clears the ITQ market (the price for which demand equals supply) for the given, fixed supply of industry quota, $\bar{Y}$. This price is equivalent to that of an auction market or an optimal tax (Anderson 1988) and implicitly assumes that all units of quota are exchanged on the market. Thus, τ^* was determined

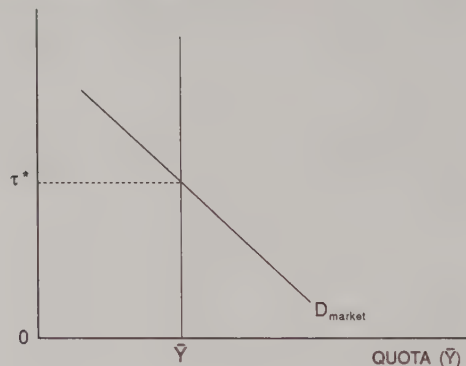


FIG. 3. Equilibrium market price for individual transferable quotas.

by evaluating equation (7) at $\bar{Y} (= \Sigma \bar{Y}_i)$. This aggregate quota $\bar{Y}$ forms the vertical (perfectly inelastic) sablefish industry quota supply curve. Figure 3 illustrates the market for one quota allocation. Six different values for τ^* were obtained, one for each $\bar{Y}$.

Shadow values from the linear programming model represent the value to the firm of an additional unit of output, including the value of the additional landed bycatch made available by relaxing the binding quota constraint. Hence, the market equilibrium price for sablefish quota can be greater than the ex-vessel sablefish price because τ incorporates the vessel's revenues from bycatch. Sample vessels were selected based upon minimum annual landings of 0.5 metric ton of sablefish rather than on a per trip basis. Some trips may have targeted more upon sablefish than other species. Hence, the contribution of bycatch to the unit rent of the sablefish quota, τ , may be overstated by the model.

2.9. Gains from Trade

Industry gains from trade are the difference between the industry rent after trade and the total industry rent before trade (but after the quota allocation), assuming all sablefish rents are valued in τ^* . Industry rent gains occur when vessels trade quota allocations $\bar{y}$, each vessel adjusting production and quota holdings to maximize quasi-rents (also called producer surplus or short-run profits; see Just et al. 1982). The total industry rent after trade is the area under the market demand curve for quota up to $\bar{Y}$. Given the linear demand curve, this area is $\tau^* \bar{Y} + 0.5[\alpha_0 + 1/N \Sigma \alpha_j D_j - \tau^*] \bar{Y} = 0.5[\tau^* + \alpha_0 + 1/N \Sigma \alpha_j D_j] \bar{Y}$. The total industry gains from trade at the competitive equilibrium market price, τ^* , are $0.5[\tau^* + \alpha_0 + 1/N \Sigma \alpha_j D_j] \bar{Y} - 0.5 \Sigma [\tau + \alpha_0 + 1/N \Sigma \alpha_j D_j] \bar{y}$ (summing over all vessels for each of the six aggregate quotas $\bar{Y}$).

3. Empirical Results and Discussion

The linear programming model was run for the six different quota allocations for each of the 30 vessels. The subsequent data generated for the simulation were six sets of τ - $\bar{y}$ pairs for the 30 vessels. Equation (6), the firm's inverse demand for quota, was then estimated by Tobit analysis as discussed in Section 2.7.

3.1. Inverse Demand for Quota

The statistical significance of the vessel size class dummies in equation (6) was assessed by a log-likelihood test. The like-

TABLE 1. Parameter estimates of vessel inverse demand curve for quota. An asterisk indicates statistically significant at 2%. Intercept is smallest vessel size class. Tobit analysis, giving maximum likelihood estimates. Log-likelihood = -1485.5.

Variable	Coefficient	t-ratio	Significance
Constant	3308.16*	2.579	0.9911
Size two dummy	-2731.54	-1.261	0.8038
Size three dummy	-991.264	-0.486	0.4838
Size four dummy	-788.081	-0.352	0.3866
Size five dummy	38628.4*	13.150	1.00
Quota	-66.214*	-2.229	0.9853

TABLE 2. Equilibrium quota market price flexibilities.

Quota allocation	Price flexibility
Initial allocation	-0.24279
90% of initial	-0.20902
80% of initial	-0.19248
70% of initial	-0.18644
60% of initial	-0.18633
50% of initial	-0.18565

lihood ratio test statistic for all dummy variables was 123.4 for four independent restrictions, indicating that the null hypothesis of no differences in unit rents among size classes could be rejected at the 0.01 level of significance. The three midsized vessel size classes have negative coefficients for the dummy variables; these coefficients were not individually statistically significant from zero but were as a group, since the chi-square statistic was 113.0 for three restrictions. Hence, the full model including all vessel size classes was retained (Table 1).

The positive sign of the statistically significant coefficients for the largest and smallest size classes indicated that these vessels have the largest unit quota rents τ (prior to exchange). The negative signs for the midsized vessels indicate inefficiency compared with the smallest and largest. Hence, the largest vessels, and possibly the smallest, should have the largest unit quota rents τ and purchase quota from the midsized vessels selling quota.

3.2. Elasticity of Market Demand for Quota

The price flexibility of τ^* , $\delta \ln \tau^* / \delta \ln \bar{Y}$, indicates the percentage change in equilibrium quota prices τ^* for a 1% change in the overall quota, $\bar{Y}$, i.e. the sensitivity of τ^* to changes in quota $\bar{Y}$. The estimated price flexibilities are inelastic, which means that a 1% increase in quota reduces τ^* by less than 1%, in this case 0.24%. Extreme short-run price variability is not expected. The price flexibility declines with tighter quotas (Table 2). An inelastic price flexibility is consistent with an elastic demand (Tomek and Robinson 1981), so that an increase in the ITQ market price by greater than 1% causes a greater than 1% decline in ITQ quantity demanded. Hence, market demand for quota is elastic.

An elastic market demand for quota suggests that total resource rents inversely vary with the level of quota, but that these proportional changes in resource rents are less than the proportional change in the aggregate quota. Hence, the fishery's economic rent can be maintained at nearly the same level over a broad range of overall quotas. This result should facilitate resource conservation, since conservative quotas could be used without a large opportunity cost of rent foregone. This suggests a conservative strategy that simultaneously protects the resource stock and insulates the industry against abrupt and

large reductions in the overall quota if new stock assessments show a sudden population decline or that previous resource assessments were in error (Squires 1991).

3.3. Equilibrium ITQ Market Price

The market equilibrium prices for the sablefish ITQ, τ^* , conditional upon the existing fleet structure and vessel sizes, are reported in Table 3 for the six alternative levels of industry quota, $\bar{Y}$. For the initial allocation, τ^* was \$3193. As expected, τ^* monotonically increased as $\bar{Y}$ decreased, peaking at \$4175.83.

The values of τ^* are relatively high and are also relatively insensitive to the size of the overall quota level (reducing overall quota by 50% resulted in only a 31% increase in equilibrium ITQ price τ^*). The ITQ should generate an important asset value to initial holders of the right, unless the rights are initially auctioned or are taxed (which transfers some or all of the economic rent from the private sector to the public sector). Moreover, the ITQ has the potential to form a barrier to entry into a fishery with otherwise relatively limited capital requirements. It might be advisable to allocate some of the initial quota to crewmembers to allow the traditional upward mobility historically enjoyed.

The value for τ^* includes the gains in quasi-rents from species other than sablefish when the binding sablefish quota is relaxed. That is, when vessels are allowed to catch more sablefish in the model, they also catch more of other species, which also contributes to their revenues. The more efficient vessels are capable of using revenues from these other species to cross-subsidize purchase of quota. This also lowers unit costs of harvesting all species by spreading out annual fixed costs over greater volume of catch. This then increases overall competitiveness, particularly since much competition is based on output price. Hence, the largest vessels and those with a more diversified harvesting strategy (e.g. they may make targeted rockfish trips) are most likely to trade for quota.

3.4. Economic Rents

The total sablefish rent for the initial allocation of quota, given the capital stock and fleet structure and prior to trade, was \$2.031 million (Table 3). This is the total rent implicit to a nontransferable quota. After exchange, total rent climbed to \$3.138 million, a 67.35% gain from the initial overall quota due to trade and increased efficiency.

The potential size of the rent also suggests ample scope for the public sector to transfer some of the rent from the private sector to the public sector. One possibility is a tax or license fee (Grafton 1992). Auction markets for the initial allocation are also possible.

The proportional gains from trade depend upon the number of vessels exchanging quota. Initially, the most and least efficient vessels trade quota, since between them they enjoy the largest disparities in unit quota rents. Hence, they realize the greatest gains from trade and are least likely to be hampered by the relatively high costs of transactions and information that occur when ITQ markets are rudimentary and thin (few buyers and sellers). When the quota progressively tightened, the proportional gains from trade dropped considerably as the disparity between the efficient and inefficient vessels narrowed.

The results nonetheless indicated substantial gains in efficiency enjoyed through exchange of quota for all quota allocations (Table 3) and the importance of a fully specified property right structure. The relatively large potential rents indicated the size of rent dissipation due to open-access and the potential rents gained through rights-based management. The expected rents may be larger than the regulatory costs of planning, implementing, monitoring, and enforcing an ITQ program for sablefish. A complete cost-benefit analysis of an ITQ program can utilize the projected rents as part of economic benefits.

The reported rents and gains from trade are the maximum possible. In practice, less trade may be observed due to thin markets, private and sequential exchange between vessels rather than all quota exchanged in the ITQ market (all quota are exchanged in the simulation), transactions and information costs, and uncertainty (Noll 1982; Hahn and Noll 1983; Lindner et al. 1989; Lindner 1990; Atkinson and Tietenberg 1991). Markets also take time to form and efficiently operate.

These relatively large gains could reflect large intervessel differences in efficiency, but they may also reflect the relatively extreme solutions often found with linear programming (Shepherd and Garrod 1980). Thus, intervessel efficiency differences may be heightened more with linear models than with models allowing for increasing marginal costs. Also, the potential rents are relatively large because they are short-run and hence exclude costs of capital and other fixed costs (e.g. insurance, moorage).

The results can be compared with the simulation results for a thornyhead transferable quota in the multispecies deepwater trawl fishery in Eureka for 1984 (Squires 1991). The proportional gains from trade in that study were generally less than 10%. The results are a bit difficult to compare because one study depends upon linear programming and maximization of quasi-rents and the other upon econometrics and maximization of revenues.

These results nonetheless suggest that a directed fishery may enjoy greater efficiency gains because of its greater flexibility in the product decision. That is, species mix decisions in a trawl fishery with joint production (multiple outputs produced by a single production process) reflect returns from several species, which must be weighed against one another. Moreover, there may well be lesser scope to alter production, to trade, and to

TABLE 3. Short-run equilibrium quota market price and total quota rent. Gains from trade are industry totals. Equilibrium market price in 1987 U.S. dollars per metric ton. Values estimated conditional upon existing capital stock.

Quota allocation	Market price for quota (\$)	Fleet quota (t)	Total economic rent prior to trade (\$)	Total optimum economic rent (\$)	Gains from trade (\$)	% gain in rent
Initial allocation	3193.79	889.87	2031074	3398956	1367822	67.35
90% of initial	3390.20	800.883	1295791	3137704	1841913	142.15
80% of initial	3586.59	711.906	2183023	2859015	675992	30.97
70% of initial	3783.02	622.909	1998154	2562782	564628	28.26
60% of initial	3979.42	533.922	1733107	2249101	515994	29.77
50% of initial	4175.83	444.935	1494112	1917946	423834	28.37

TABLE 4. Number of quota purchases for alternative quota allocations as a percentage of initial quota holding.

Vessel size class	100%	90%	80%	70%	60%	50%
1 (<i>n</i> = 14)	0	1	1	1	1	1
2 (<i>n</i> = 5)	0	0	0	0	0	2
3 (<i>n</i> = 5)	1	1	1	1	1	1
4 (<i>n</i> = 4)	0	0	1	1	1	1
5 (<i>n</i> = 2)	1	2	2	2	2	2
Total (<i>n</i> = 30)	2	4	5	5	5	7

TABLE 5. Number of quota sales for alternative quota allocations as a percentage of initial quota holding.

Vessel size class	100%	90%	80%	70%	60%	50%
1 (<i>n</i> = 14)	14	13	13	13	13	13
2 (<i>n</i> = 5)	5	5	5	5	5	3
3 (<i>n</i> = 5)	4	4	4	4	4	4
4 (<i>n</i> = 4)	4	4	3	3	3	3
5 (<i>n</i> = 2)	1	0	0	0	0	0
Total (<i>n</i> = 30)	28	26	25	25	25	23

enjoy gains from trade in a trawl fishery than in a directed long-line fishery.

3.5. Concentration of Quota and Limitations to Quota Exchange

In practice, a well-formed price signal τ^* , such as that formed in the simulation, may not develop through extensive quota trade in competitive, well-functioning quota markets. In this case, the price signal does not fully and accurately convey the information for the most efficient resource allocation possible. A competitive market, with large numbers of buyers and sellers who actively trade permits, and a market clearing price do not necessarily develop (Noll 1982; Hahn and Noll 1982; Lindner et al. 1989; Lindner 1990; Atkinson and Tietenberg 1991). Even if the quota market is not concentrated (i.e. quota is not held by relatively few firms), the number of vessels deciding to trade their initial allocation of quota may be too few, the transactions may be strictly sequential, bilateral, and private (i.e. not appearing in the market), uncertainty may be important, or the unit size of quota may be too large, so that transactions are infrequent. In turn, infrequent trades and possibly highly variable price signals can undermine efficient choices of production, investment, and quota exchange and reduce the maximum expected economic rent and arbitrage efficiency. Along these lines, Lindner et al. (1989) and Lindner (1990) reported a widely dispersed price signal, relatively thin markets, and sequential and bilateral ITQ trade in New Zealand and provided a detailed discussion of the likely determinants of quota exchange and market performance.

Tables 4 and 5 report the pattern of quota purchases and sales in the simulation, in which all vessels have exchanged quota. Most vessels sell quota at all allocations, particularly the smallest vessels, and only a few buy, generally the largest vessels.

Vessels that sell quota sell all of it when trade is simulated with a linear model, since the unit quota rent τ is constant over all trade levels. Vessels compare this value τ with the ITQ market equilibrium price τ^* and if $\tau^* > \tau$, they sell all of their quota. In a model with upward sloping supply and marginal cost curves for sablefish, τ progressively declines (grows) as vessels buy (sell) quota. In this case, τ , equalized at the last unit exchanged among all vessels, can occur with vessels trading lesser amounts of quota than with the linear model. Hence,

more balanced trade patterns can be expected. This issue is under research.

The simulated quota exchange indicates potential for concentration of quota by only a few vessels. The quota market risks becoming thin, noisy, and hampered by noncompetitive forces, potentially requiring limits to quota transfer and concentration—even when quota is initially allocated through auction markets.

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References

- ANDERSON, J. E. 1988. The relative inefficiency of quotas. MIT Press, Cambridge, MA.
- ANDERSON, L. G. 1991. A note on the market power in ITQ fisheries. *J. Environ. Econ. Manage.* 21: 291–296.
- ANONYMOUS. 1991. Fish market reports. *Commer. Fish. News* 11(18): 18.
- ATKINSON, S. E., AND T. H. TIETENBERG. 1991. Market failure in incentive-based regulation: the case of emissions trading. *J. Environ. Econ. Manage.* 21: 17–31.
- BAUMOL, W., AND W. OATES. 1971. The use of standards and pricing for protection of the environment. *Swed. J. Econ.* 7: 42–54.
- BROWN, B., J. BRENNAN, AND J. PALMER. 1979. Linear programming simulations of the effects of bycatch on the management of mixed species fisheries off the northeastern coast of the United States. *Fish. Bull.* 76: 851–860.
- CAMPBELL, H. 1990. Resource rent and fishery management. Paper presented to the Australian and New Zealand Southern Trawl Fisheries Conference, Melbourne, May 1990.
- CHRISTY, F. JR. 1973. Fisherman's catch quotas. Occasional Paper No. 19, Law of the Sea Institute, University of Rhode Island, Kingston, RI.
- CLARK, C. 1976. Mathematical bioeconomics. Wiley, New York, NY.
- COPES, P. 1986. A critical review of the individual quota in fisheries management. *Land Econ.* 62: 278–291.
- DALES, J. 1968. Pollution, property and prices. University of Toronto Press, Toronto, Ont.
- GEEN, G., R. BROWN, AND S. PASCOE. 1990. Management policies for the south-east trawl fishery: an economic analysis. Invited paper to the 34th Annual Conference of the Australian Agr. Economics Society, University of Queensland, Brisbane, 13–15 Feb. 1990.
- GEEN, G., AND M. NAYER. 1989. Individual transferable quotas in the southern bluefin tuna fishery: an economic appraisal. Rights based fishing. Kluwer Academic Publishers, Boston, MA.
- GRAFTON, R. Q. 1992. Rent capture in an individual transferable quota fishery. *Can. J. Fish. Aquat. Sci.* 49: 497–503.
- HAHN, R. W. 1984. Market power and transferable property rights. *Q. J. Econ.* 99(1984): 753–765.
- HAHN, R. W., AND R. G. NOLL. 1983. Designing a market for tradable emissions permits, p. 119–146. *In* E. F. Joeres and D. H. Martin [ed.] *Buying a better environment*. University of Wisconsin Press, Madison, WI.
- HAYNES, J., AND S. PASCOE. 1988. A policy model of the northern prawn fishery. Occasional Paper 103, Australian Bureau of Agricultural and Resource Economics, Canberra, Australia.
- HUPPERT, D., AND D. SQUIRES. 1987. Potential economic benefits and optimum fleet size in the Pacific Coast trawl fleet. *Mar. Resour. Econ.* 3: 297–318.
- JUST, R., D. HUETH, AND A. SCHMITZ. 1982. Applied welfare economics and public policy. Prentice-Hall, Englewood Cliffs, NJ.
- LAU, L. 1976. A characterization of the normalized restricted profit function. *J. Econ. Theory* 12: 131–163.
- LINDNER, R. 1990. Something fishy in the ITQ market. Paper presented to the Australian Agricultural Economics Society, 34th Annual Conference, University of Queensland, 13 to 15 Feb. 1990.
- LINDNER, R., H. CAMPBELL, AND G. BEVIN. 1989. Rent generation during the transition to a managed fishery: the case of the New Zealand ITQ system. University of Western Australia, Perth, Australia.
- LOVELL, C., AND P. SCHMIDT. 1988. A comparison of alternative approaches to the measurement of productive efficiency. *In* A. Dogramaci and R. Fare [ed.] *Applications of modern production theory: efficiency and productivity*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

- MADDALA, G. S. 1985. Limited-dependent and qualitative variables in econometrics. Cambridge University Press, Cambridge.
- MEURIOT, E., AND J. GATES. 1983. Fishing allocations and optimal fees: a single- and multilevel programming analysis. *Am. J. Agric. Econ.* 65: 711–721.
- MISIOLEK, W., AND H. ELDER. 1989. Exclusionary manipulation of markets for pollution rights. *J. Environ. Econ. Manage.* 16: 156–166.
- MOLONEY, D. G., AND P. H. PEARSE. 1979. Quantitative rights as an instrument for regulating commercial fisheries. *J. Fish. Res. Board Can.* 36: 859–866.
- MONTGOMERY, W. 1972. Markets in licenses and efficient control programs. *J. Econ. Theory* 5: 395–418.
- MUSE, B., AND K. SCHELLE. 1989. Individual fisherman's quotas: a preliminary review of some recent programs. *Alaska Commer. Fish. Entry Comm. Pap.* 89-1: 122 p.
- NATURAL RESOURCES CONSULTANTS. 1988. Report on pot and longline fishing for groundfish off California, Oregon and Washington. Natural Resources Consultants, Seattle, WA. 10 p.
- NEARY, J., AND K. ROBERTS. 1980. The theory of household behavior under rationing. *Eur. Econ. Rev.* 13: 24–42.
- NOLL, R. 1982. Implementing marketable emissions permits. *Am. Econ. Rev.* 72: 120–124.
- OVERHOLTZ, W. 1985. Managing the multispecies otter trawl fisheries of Georges Bank with catch optimization methods. *N. Am. J. Fish. Manage.* 5: 252–260.
- SCHULZE, W., AND R. D'ARGE. 1974. The Coase proposition, information constraints, and the long-run equilibrium. *Am. Econ. Rev.* 64: 763–772.
- SCOTT, A. 1986. Catch quotas and shares in the fishstock as property rights. *In* E. Miles et al. [ed.] *Natural resource economics and policy applications*. University of Washington Press, Seattle, WA.
- SHEPHARD, J. G., AND D. J. GARROD. 1980. Modelling the response of a fishing fleet to changing circumstances, using cautious nonlinear optimization. *J. Cons. Int. Explor. Mer* 39: 231–238.
- SIEGEL, R., J. MUELLER, AND B. ROTHCHILD. 1979. A linear programming approach to determining harvesting capacity. *Fish. Bull.* 77: 425–433.
- SISSINWINE, M. P., AND P. M. PACE. 1992. ITQs in New Zealand: the era of fixed quotas in perspective. *Mar. Resour. Econ.* 90: 147–160.
- SQUIRES, D. 1990. Individual transferable quotas: theory and an application. *Southwest Fish. Sci. Cent. Admin. Rep.* LJ-90-16.
1991. The potential effects of individual transferable quotas in the Eureka deepwater trawl fishery. *Southwest Fish. Sci. Cent. Admin. Rep.* LJ-91-13.
- SQUIRES, D., M. ALAUDDIN, AND J. KIRKLEY. 1992. The potential effects of individual transferable quotas in the fixed gear sablefish fishery. *Southwest Fish. Sci. Cent. Admin. Rep.* LJ-92-08.
- SQUIRES, D., AND J. KIRKLEY. 1991. Production quota in multiproduct Pacific fisheries. *J. Environ. Econ. Manage.* 21: 109–126.
- TOMEK, W., AND K. ROBINSON. 1981. *Agricultural product prices*. 2nd ed. Cornell University Press, Ithaca, NY.
- WEITZMAN, M. 1974. Prices vs. quantities. *Rev. Econ. Stud.* 41: 50–65.

Role of Predatory Fish in Community Dynamics of an Ephemeral Stream

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In enclosed runs in a third-order ephemeral stream in west-central Kentucky, the effects of predatory sunfish (*Lepomis*) on benthic macroinvertebrates, benthic algae, and detritivory were compared (1) before stream intermittence, (2) after stream intermittence when transport was restricted, and (3) between substrata offering differential cover from fish predation. Ambient fish densities had little effect on total macrobenthic densities and processes on lower trophic levels before intermittence. Fish modestly affected macroinvertebrate densities after intermittence, when surface exchange of prey was interrupted by sections of dry stream. Among substrata, fish influenced macroinvertebrates on bedrock, but not on stony, coarse substrata. Densities of two taxa were significantly affected by fish, and this significantly altered the relative abundance of functional feeding groups in enclosures on bedrock by increasing the proportion of invertebrate predators in fish treatments. Macroinvertebrate densities in microhabitats on both substrata were not affected by fish presence. Dense growths of the stalked diatom *Cymbella* generally covered microhabitats and added structural complexity, particularly to bedrock surfaces. Unobstructed, natural migration of prey in the large (40 m²) fence-like enclosures (versus containers), ample refuge space, and low natural densities of fish were important in minimizing fish effects in enclosures.

Dans des passages cloisonnés d'un cours d'eau intermittent de troisième ordre, dans la région centre-ouest du Kentucky, nous avons comparé les effets du crapet prédateur (*Lepomis*) sur les macroinvertébrés benthiques, les algues benthiques et les détritivores 1) avant l'intermittence du cours d'eau, 2) après l'intermittence du cours d'eau lorsque le transport était interrompu et 3) entre les substrats offrant un abri différentiel contre la prédation des poissons. Les densités de poissons ambiantes ont eu peu d'effets sur les densités macrobenthiques totales et sur les processus des niveaux trophiques inférieurs avant l'intermittence. Les poissons ont eu une influence très modérée sur les densités de macroinvertébrés après l'intermittence, lorsque l'échange de surface des proies était interrompu par des sections de cours d'eau asséchées. En ce qui concerne les substrats, les poissons ont eu une influence sur les macroinvertébrés sur l'assise rocheuse, mais pas sur les substrats pierreux et grossiers. Les densités de deux taxons ont été significativement altérées par les poissons, ce qui a eu une influence significative sur l'abondance relative des groupes fonctionnels trophiques dans les zones cloisonnées sur l'assise rocheuse en augmentant la proportion des invertébrés prédateurs dans les traitements. Les densités de macroinvertébrés dans les microhabitats sur les deux substrats n'ont pas été modifiées par la présence des poissons. Une croissance dense de la diatomée pédonculée *Cymbella* recouvrait en général les microhabitats et ajoutait une complexité structurale, en particulier pour les surfaces d'assise rocheuse. La migration naturelle et non entravée des proies dans les vastes (40 m²) enceintes délimitées par des clôtures (par opposition aux contenants), le vaste espace de refuge et les faibles densités naturelles de poissons ont contribué de manière importante à minimiser les effets des poissons dans les enceintes.

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The role of fish predators in regulating the trophic and community structure in streams continues to be debated. Some studies show that fish predators can significantly alter species assemblages and reduce richness and/or densities of benthic macroinvertebrate prey (Griffiths 1981; Bowlby and Roff 1986; Power 1990); others show no such effects (Allan 1982; Flecker 1984; Flecker and Allan 1984; Culp 1986; Reice and Edwards 1986). Further, the few studies that have examined the indirect effects of stream fishes on species and processes occurring two or more trophic levels lower in the food web also show effects

in some streams (Power and Matthews 1983; Power 1990) but not in others (Hill and Harvey 1990; Reice 1991).

The degree to which fish predators affect the prey of stream communities depends at least in part on the substratum and flow regime of the particular stream or stream habitat. For example, fish have been shown to significantly reduce the densities of benthic macroinvertebrate prey that occur on fine substrata (i.e. silt, sand) or on bare bedrock with little refuge space (Gilliam et al. 1989; Holomuzki and Hoyle 1990; Holomuzki and Short 1990) and in slow-flowing water (Hemphill and Cooper 1984; Schlosser and Ebel 1989). Yet, such effects are generally not observed when prey live on stony, coarse substrata in fast-

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flowing water (e.g. Allan 1982; Culp 1986). Thus, ample refuge space and transport from other habitats, particularly in patchy environments, may effect the ability of prey to avoid the effects of predation and to maintain populations within the habitat mosaic.

This paper describes a series of field experiments designed to determine the conditions in which predatory longear (*Lepomis megalotis*) and green sunfish (*L. cyanellus*) affect lower trophic levels in a small, ephemeral stream in west-central Kentucky, USA. These fish occur in all major drainages in Kentucky (Clay 1962) and are benthic feeders, eating predominantly isopods, mayflies, and chironomids (Minckley 1963; Holomuzki and Short 1988, 1990). Sunfish densities were manipulated with large enclosures to determine whether sunfish affected benthic macroinvertebrates, benthic algae, or detritivory (1) before stream intermittence, (2) after intermittence when transport of prey was restricted, and (3) on different substrata offering differential cover from predation. We predicted that fish effects would be greatest after flow was interrupted by sections of dry stream because surface exchange (i.e. drift) into fish areas would be greatly restricted or lacking when riffles dry and pools and runs become isolated. Restrictions in immigration/emigration rates are known to enhance predatory effects on prey species (Cooper et al. 1990). We also predicted that fish predators would reduce densities of macroinvertebrates on substrata offering little refuge space (e.g. exposed bedrock, silt/sand) more so than on stony substrata. In situations where fish may directly affect benthic macroinvertebrate densities and assemblages, fish may also indirectly affect processes at lower trophic levels, such as algal growth and assemblages, rates of detrital breakdown, and nutrient dynamics.

Methods

Study Site

The study was done in Hart's Run, an ephemeral third-order tributary of Wilson Creek in Bullitt County, Kentucky. The stream drains a forested catchment within Bernheim Forest (37°50'N, 85°35'W), a pristine nature preserve 50 km southeast of Louisville. The entire stream is shaded by an overstory dominated by sycamores (*Platanus occidentalis*) and white oaks (*Quercus alba*). Discharge in May is about 0.07 m³/s but drops to 0.02 m³/s by June. The stream typically begins to dry by mid-June, particularly in upstream reaches, and all but the deepest pools are dry by early August. Stream morphology is best defined as gravelbed (Resh et al. 1988) with cobble or exposed areas of dolomitic limestone bedrock in runs. Runs occupy nearly all of the total stream length, while pools and riffles represent $\leq 10\%$ of the total length. Chemically the stream is oligotrophic. Early spring concentrations of nitrate-nitrogen (NO₃-N) and orthophosphate (PO₄-P) average about 6.0 and 3.0 $\mu\text{g/L}$, respectively (Stevenson et al. 1991).

The benthic algae are dominated by small *Synedra* spp. and *Achnanthes minutissima* in spring (Stevenson et al. 1991), but *Cymbella* is predominant in early summer. The invertebrate community is dominated by mayflies, particularly *Paraleptophlebia guttata*, and chironomids in spring (J. Holomuzki, unpubl. data). Relatively few fish species inhabit the stream. Longear and green sunfish and grass pickerel (*Esox americanus vermiculatus*) are by far the most abundant fish species. Darters, sculpins, and cyprinids are common only near the confluence of Wilson Creek. Fish densities are ≈ 8 ind./10 m² 100–500 m from the confluence of Wilson Creek but drop to ≤ 1 ind./10 m² beyond about 1000 m.

Experimental Design

Fish densities were manipulated by enclosing and dividing runs. Enclosures were 40 m² and constructed of galvanized hardware cloth. They extended from bank to bank and were supported by steel stakes hammered into the substrata and/or by boulders. When feasible, both the upstream and downstream sides of the enclosures were sunk ≈ 20 cm into the substratum to prevent fish from escaping and/or entering. Mesh size of the hardware cloth (6.3 mm) was large enough to readily allow immigration and emigration of most benthic invertebrates but small enough to exclude adult fish. Each enclosure was partitioned longitudinally by hardware cloth running parallel with the current and dividing each enclosure into 20-m² halves. We covered the dividing partition above and below the water surface and ≈ 20 cm below the substratum with 100% polypropylene shade screening (eighteen 0.04-mm meshes/cm) to restrict macroinvertebrates from moving between enclosure halves. We felt the size and fence-like design of enclosures more closely simulated natural conditions than scaled-down experimental containers (see Walde and Davies 1984; Cooper et al. 1990) and allowed for a representative prey community and for unobstructed surface and substratum recruitment of prey.

To enhance the power of our statistical analyses and to detect fish effects, we spaced the enclosures widely and amply replicated their numbers. Enclosures were spaced 10–300 m apart over a 1600-m length of Hart's Run. The enclosure nearest to the confluence of Hart's Run and Wilson Creek was 200 m away. Care was taken to choose sites with similar substrate composition, canopy cover, current velocity (≈ 10 cm/s), and depth (35–50 cm) between enclosure halves because these factors can influence macroinvertebrate and algal densities and assemblages (e.g. Robinson and Rushforth 1987; Feminella et al. 1989). Substrate composition based on particle size (Cummins 1962) in runs was, on average, about 17% sand, 26% gravel, 26% pebble, and 21% small cobble. Debris was cleaned from the sides of enclosures once or twice weekly to prevent clogging and the restriction of surface immigration/emigration of benthos.

We stocked two fish (7–15 cm standard length), either longear or green sunfish, in only one half of each enclosure. This density corresponds with natural densities ($\approx 1/10$ m²) of sunfish in slow runs in this stream. The enclosure half that received fish was determined by coin toss. We electroshocked within the enclosures every 1–2 wk during the study to monitor fish presence.

1989 Sampling Protocol

We constructed 11 enclosures in stony runs from 2 to 10 May. Fish were stocked in enclosures on 26 May to determine the effects of fish before and after stream intermittence. Only longear sunfish were used in 1989. We sampled benthic macroinvertebrates in both halves of all enclosures on 16–19 May before fish stocking, on 20–21 June as the stream became intermittent, and on 7–12 July, about 20 d after stream intermittence. The final sampling was done just before runs with enclosures began to dry. Macroinvertebrates were sampled within a solid-wall plastic box (370 cm²) with open top and bottom, placed firmly on the stream bottom. All animals were removed with a dip net (80- μm mesh) from the enclosed substratum to a depth of 3–5 cm and immediately preserved in 70% ethanol. A total of five to seven samples were taken from each enclosure half along a 0.5-m-wide belt transect. Several microhabitats were sampled: sand/silt, gravel, pebble/small cobble,

and leaf litter. We tried to sample the same number and kinds of microhabitats per enclosure half to avoid a density bias from microhabitat influences. Invertebrates were separated from substrates by sugar flotation (Lind 1974) and hand-sorting. All invertebrates were identified to genus and species, except for chironomids which were identified to family. Counts of each invertebrate taxon from samples for each enclosure half were averaged for density determinations.

We estimated algal standing crop by placing nine unglazed ceramic tiles (each 29.1 cm²) used as substrata for algal colonization (Stevenson 1984a) in each enclosure half on 30 May. Three tiles were sampled on 12 June, 13 d after colonization during preintermittence, and another three on 3 July, 34 d after colonization during postintermittence. Algae were scraped with a small brush and rinsed from tiles with distilled water into whirl-pak bags. Samples were immediately stored on ice in the field and then taken back to the laboratory and frozen. Chlorophyll *a* from samples was extracted in buffered acetone at 5°C for 24 h within 2 wk of collection. Pigment concentration was analyzed fluorometrically (APHA 1985) and expressed in grams chlorophyll *a* per square centimetre. The average chlorophyll *a* concentration from the three samples per enclosure half was used to measure algal standing crop for each sampling period.

Leaf processing was quantified following methods in Arsuffi and Suberkropp (1985). We cut leaf disks 18 mm in diameter from conditioned sycamore leaves collected from Hart's Run. All disks were similar in toughness as assessed by penetrometer. Each disk was secured between two 5 × 5 cm pieces of galvanized hardware cloth (6.3-mm mesh). Eight disks were placed in each enclosure half on 2 June. Degree of processing was assessed on 21 June and on 7 July. On a continuum basis, no and complete skeletonization were scored as 5 and 0, respectively. The mean scores from the eight disks per enclosure half were used to compare leaf breakdown among fish treatments.

1990 Sampling Protocol

In this year, we tested for differences in fish effects on bedrock and coarse substrata. From 7 to 14 May, we constructed a total of 17 enclosures; eight were built on exposed bedrock and nine on stony substrata. On 4 June, 10 enclosures were stocked solely with longear sunfish, while another seven contained both longear and green sunfish. We could not obtain enough longears from Hart's Run in 1990 to stock all enclosures. Macroinvertebrates were sampled as in 1989 in all enclosures on 17–19 May before fish stocking and on 1–12 July, 28–39 d after fish stocking, as some runs with enclosures began to dry up.

Differences in algal community structure between fish and fishless treatments were assessed by measuring chlorophyll *a*, ash-free dry mass (AFDM), cell densities, and species composition. Six ceramic tiles, used as substrata for algal colonization, were placed in each enclosure half on 11 June. Tiles were collected from enclosures between 1 and 12 July. Three randomly chosen tiles from each enclosure half were scrapped, and each was analyzed for chlorophyll *a* as in 1989. The other three tiles were scraped into one composite sample for each enclosure half and preserved in M3 (APHA 1985). Algae from composite samples were mounted on microscope slides in syrup (Stevenson 1984b) and ~200 cells were counted to determine species composition and cell density. The material remaining in composite samples after slide preparation was processed to determine AFDM (APHA 1985).

To assess leaf litter breakdown, plastic litter bags (7-mm mesh) measuring 15 × 15 cm were filled with 2.0 g of dry sycamore leaves. Only leaves that had fallen over the same 48-h interval in the autumn of 1989, and thus presumably similar in toughness, were selected. After collection, leaves were stored at 0°C. Seven days before placement into bags, leaves were thawed and allowed to air-dry. Four litter bags were placed on the bottom of each enclosure half on 11 June and tied to upstream enclosure sides to prevent displacement from discharge fluctuations. All bags were removed from enclosures between 1 and 12 July. After air-drying, leaves were oven-dried for 24 h at 60°C and then immediately weighed. Mean percent loss of dry mass was calculated for each enclosure half.

Water samples were collected before (19–21 May) and after (1–12 July) fish stocking to determine levels of NO₃-N and PO₄-P, key nutrients that potentially limit algal growth in Hart's Run (Stevenson et al. 1991). Three samples per enclosure half were collected with a 60-mL polyurethane syringe and filtered (Gelman GN-6, 0.45-μm pore size) into acid-rinsed polypropylene bottles in the field. All samples were immediately placed on ice, returned to the laboratory, and frozen until analyzed. NO₃-N and filtrable-PO₄-P were measured using the cadmium reduction and ascorbic acid method (APHA 1985), respectively, with a dual-channel Technicon AutoAnalyzer II.

Finally, we stomach-flushed (Zerba 1989) a total of 23 fish in late June and early July to determine if fish were indeed feeding while enclosed. Contents were preserved in 70% ethanol, and prey items were counted.

Analytical Procedures

Paired sample *t*-tests were used to test for differences in benthic macroinvertebrate and algal community characteristics, leaf breakdown, and nutrient concentrations between fish and fishless treatments. Split-plot ANOVA were used to test for interactive effects between predator and substrate treatments in the 1990 experiment. Absolute densities of benthic macroinvertebrates and algae and other measures of algal standing crop were log (*X* + 1) transformed to homogenize variances. Relative densities (percent) and percent leaf breakdown were arcsine-transformed before analysis. We used Kendall's coefficient of rank correlation (Sokal and Rohlf 1981) to compare relative densities of invertebrates between enclosure halves prior to fish stocking. Finally, *G*-tests were used to compare proportions of functional feeding groups in enclosures between fish treatments.

Results

Trophic Characteristics before Fish Stocking

Total invertebrate densities and species richness did not differ between enclosure halves prior to fish stocking in both years (Table 1). Additionally, relative densities of the more common benthic invertebrates were similar among enclosure halves (Table 2). Ranks of relative densities of these taxa between enclosure halves to receive (TR) and not to receive (NTR) fish (Table 2) were also similar (Kendall's tau = 0.782, *n* = 11, *P* < 0.01).

Mayflies and chironomids were the predominant invertebrates in the stream in late May. The leptophlebiid mayfly *P. guttata* was the most abundant invertebrate. Although chironomid species were lumped in our analyses, many (~50%) were herbivorous Diamesinae, but roughly 10% were predaceous Tanytopodinae.

TABLE 1. Mean (± 1 SE) total macroinvertebrate densities (ind./m²) in May 1989 and 1990 in enclosures prior to fish stocking. TR = to receive fish; NTR = not to receive fish. In all cases, $n = 11$ for 1989 and $n = 17$ for 1990. NS = not significant at $\alpha = 0.05$ (paired sample t -tests).

	TR	NTR
1989		
Total density	670 $\pm$ 59	619 $\pm$ 67 NS
1990		
Total density	1130 $\pm$ 190	976 $\pm$ 188 NS
Species richness per enclosure	9.6 $\pm$ 0.6	8.8 $\pm$ 1.1 NS

TABLE 2. Mean (± 1 SE) relative densities (%) of the common invertebrate taxa in enclosures prior to fish stocking in May 1990. TR = to receive fish; NTR = not to receive fish.

Taxon	TR	NTR
<i>Paraleptophlebia guttata</i> (Ephemeroptera)	51.1 $\pm$ 6.0	49.4 $\pm$ 5.0
Chironomids (Diptera)	25.2 $\pm$ 4.1	31.6 $\pm$ 4.3
<i>Hydroporus blanchardi</i> larvae (Coleoptera)	6.0 $\pm$ 1.0	5.4 $\pm$ 0.9
<i>Stenonema femoratum</i> (Ephemeroptera)	3.6 $\pm$ 1.1	3.6 $\pm$ 1.1
<i>Baetis intercalaris</i> (Ephemeroptera)	2.5 $\pm$ 0.7	2.4 $\pm$ 0.6
<i>Lirceus fontinalis</i> (Isopoda)	2.2 $\pm$ 0.7	2.0 $\pm$ 0.6
<i>Dannella litt</i> a (Ephemeroptera)	2.1 $\pm$ 0.6	1.7 $\pm$ 0.7
<i>Palmacorixa buenoi</i> (Hemiptera)	2.0 $\pm$ 1.5	0.1 $\pm$ 0.1
<i>Tipula</i> sp. (Diptera)	1.0 $\pm$ 0.7	0.3 $\pm$ 0.2
<i>Utaerla</i> sp. (Plecoptera)	0.8 $\pm$ 0.3	0.5 $\pm$ 0.1
<i>Hexatoma</i> sp. (Diptera)	0.2 $\pm$ 0.1	0.7 $\pm$ 0.3
Others	3.3 (20 taxa)	2.3 (13 taxa)

Nutrient concentrations were also similar among enclosure halves in 1990. Based on analyses from seven randomly chosen enclosures, mean (± 1 SE) NO₃-N values between TR and NTR enclosure halves were 21.35 $\pm$ 4.44 and 16.55 $\pm$ 5.48 μ g/L, respectively, and these means did not differ significantly ($t = 0.663$, $df = 6$, $P > 0.50$). Mean PO₄-P levels were <1 μ g/L for both TR and NTR halves.

Fish Effects before and after Stream Intermittence

Total macroinvertebrate densities, algal chlorophyll a , and leaf breakdown were similar among fish and fishless enclosures in 1989 before (May to mid-June) and after (mid-June to mid-July) stream intermittence (Table 3). Thus, fish did not affect benthic organisms or processes in enclosures even when surface recruitment was presumably restricted by stream drying. Fur-

ther, mean (± 1 SE) macroinvertebrate densities outside enclosures about 350 m upstream (between enclosures 1 and 2) on 23 June were 645 $\pm$ 70 ($n = 11$ samples), indicating that there were no enclosure effects on macroinvertebrate densities.

Two enclosures in 1989 did not consistently hold fish. Therefore, data on trophic characteristics from these enclosures were deleted from analyses.

Fish Effects on Bedrock and Coarse Substrata

Fish had little effect on lower trophic levels in enclosures regardless of substratum. Total invertebrate densities and species richness, algal chlorophyll a , AFDM, and densities and leaf breakdown and NO₃-N and PO₄-P concentrations did not differ among fish and fishless enclosures on bedrock and coarse substrata in 1990 (Table 4). As in May, mayflies and chironomids were the numerically dominant invertebrates in July on both substrata. Chironomids were, however, more abundant than mayflies, and *Stenonema femoratum* (Heptageniidae) and *Centroptilum* sp. (Baetidae) had replaced *P. guttata* as the predominant mayflies by mid-June. Other mayflies present in summer were the baetids *Choroterpes* sp. and *Callibaetis* sp., the ephemereiid *Dannella litt*a, and the leptophlebiid *Leptophlebia* sp.

Fish presence had no significant effects on the densities of any macroinvertebrates in enclosures on coarse substrata, but did significantly affect the densities of two taxa on exposed bedrock (Table 5). Larval *Hydroporus blanchardi* (Dytiscidae) were significantly more dense in enclosures with fish than without, while *Leptophlebia* sp. mayfly nymphs were significantly more abundant in fishless enclosures than in those containing fish. The differential *H. blanchardi* distributions translated into a significantly greater proportion of invertebrate predators in fish treatments (29%) than in fishless treatments (14.5%) in enclosures on bedrock ($G = 126.7$, $df = 1$, $P < 0.001$). Proportions of functional feeding groups in enclosures on coarse substrata, however, were not different between fish treatments ($G = 0.27$, $df = 2$, $P > 0.75$). No significant ($P > 0.05$) interactive effects of fish and substrate type were observed on total macroinvertebrate density.

Densities of macroinvertebrates differed among microhabitats in enclosures on bedrock, but not on coarse substrata (Table 6). Densities were significantly greater in leaf litter than in other microhabitats, but only in fishless treatments. However, there were no significant ($P > 0.05$) interactions between densities in microhabitats and fish presence on either substratum.

Fish presence also had no effect on the dominant species of the benthic algal community, whether on bedrock or coarse substrata (Table 7). Diatoms comprised 99% of the algae on tile

TABLE 3. Mean (± 1 SE) total invertebrate densities (ind./m²), leaf skeletonization, and algal standing crop (μ g Chl a /cm²) in fish and fishless enclosures before and after stream intermittence in 1989. In all cases, $n = 9$.

Trophic characteristic	Fish	No fish	t	P
Before intermittence				
Total invertebrate density	712 $\pm$ 135	736 $\pm$ 106	-0.68	>0.50
Leaf skeletonization	4.9 $\pm$ 0.03	4.9 $\pm$ 0.05	0.36	>0.50
Chlorophyll a	0.364 $\pm$ 0.159	0.252 $\pm$ 0.490	0.52	>0.50
After intermittence				
Total invertebrate density	511 $\pm$ 97	555 $\pm$ 108	-0.14	>0.50
Leaf skeletonization	4.9 $\pm$ 0.03	4.8 $\pm$ 0.04	2.14	>0.05
Chlorophyll a	1.416 $\pm$ 0.226	1.663 $\pm$ 0.448	-0.50	>0.50

TABLE 4. Mean (± 1 SE) total macroinvertebrate densities (ind./m²), species richness per enclosure, chlorophyll *a* ($\mu\text{g}/\text{cm}^2$), AFDM (mg/cm^2), algal densities (10^3 cells/cm²), leaf breakdown (%), and NO₃-N and PO₄-P concentrations ($\mu\text{g}/\text{L}$) in enclosures on bare bedrock and coarse substrata in fish and fishless treatments in 1990. In all cases, $n = 8$ and $n = 9$ for bedrock and coarse substrates, respectively.

Trophic characteristic	Fish	No fish	<i>t</i>	<i>P</i>
Bedrock				
Macroinvertebrates				
Total density	1075 $\pm$ 187	821 $\pm$ 123	1.365	>0.20
Species richness	12.2 $\pm$ 0.6	10.6 $\pm$ 0.7	2.031	>0.05
Algae				
Chlorophyll <i>a</i>	0.362 $\pm$ 0.120	0.519 $\pm$ 0.152	-0.893	>0.20
AFDM	1.322 $\pm$ 0.208	1.057 $\pm$ 0.304	0.933	>0.20
Density densities	154 $\pm$ 37	165 $\pm$ 35	-0.401	>0.50
Others				
Leaf breakdown	13.6 $\pm$ 1.7	13.1 $\pm$ 1.9	0.587	>0.50
NO ₃ -N	21.05 $\pm$ 3.60	21.04 $\pm$ 2.71	0.007	>0.50
PO ₄ -P	1.2 $\pm$ 0.1	1.3 $\pm$ 0.1	-0.519	>0.50
Coarse substrata				
Macroinvertebrates				
Total density	1087 $\pm$ 156	940 $\pm$ 106	0.874	>0.50
Species richness	13.7 $\pm$ 0.4	13.0 $\pm$ 1.1	0.419	>0.50
Algae				
Chlorophyll <i>a</i>	0.384 $\pm$ 0.147	0.635 $\pm$ 0.285	-0.341	>0.50
AFDM	1.100 $\pm$ 0.202	0.676 $\pm$ 0.153	2.209	>0.05
Density	182 $\pm$ 39	274 $\pm$ 59	-0.920	>0.20
Others				
Leaf breakdown	14.7 $\pm$ 0.8	15.0 $\pm$ 1.4	-0.083	>0.50
NO ₃ -N	15.83 $\pm$ 2.17	19.95 $\pm$ 3.31	-2.074	>0.05
PO ₄ -P	1.1 $\pm$ 0.2	1.2 $\pm$ 0.3	-0.432	>0.50

substrates. *Achnanthes minutissima*, *Cymbella cistula*, *C. minuta*, and *Synedra tenera* were the most abundant species. Abundances of these diatoms varied, although not significantly, among substrates. Absolute densities of *A. minutissima* and *S. tenera* tended to be higher on tiles in coarse substrates, while *C. minuta* was more dense on tiles on bedrock. Interactive effects of fish and substratum type were insignificant on both benthic algal standing crop and species composition.

Fish were feeding on benthic organisms while in enclosures (Fig. 1). Of the 23 fish that were stomach-flushed, 20 contained food items. The major prey items of fish regardless of enclosure substratum were chironomids and mayflies, the predominant macroinvertebrates in the stream in summer. The crayfish *Orconectes rusticus* was eaten by fish only in enclosures on coarse substrata. Fish, on average, contained only five prey items, indicating that feeding was not intense just prior to stream drying. All enclosures successfully retained fish this year.

Food Web

Enclosure food webs consisted of three trophic levels when fish were absent and four levels when fish were present (Fig. 2). A key result was that fish presence did not affect the composition of the first three levels. In general, the first trophic level included algae and detritus with associated bacteria and microflora. The predominant algae were diatoms. The filamentous green algae, *Spirogyra*, *Zygonema*, or *Mougeotia*, was scarce. Diamesinae chironomids, mayflies, and crustaceans (e.g. isopods, amphipods, crayfish), snails (*Physa*, *Gonobasis*), and craneflies (*Tipula*) were on level two. Predaceous diving beetles (Hydroporus) odonates (*Lanthus*, *Perithemus*, *Hetaerina*), craneflies (*Hexatoma*, *Pilaria*), and alderflies (*Sialis*) were the predominant predators on level three.

Interestingly, no major change in the general organization of the food web was observed from spring to summer. Chironomids and mayflies and the dytiscid *H. blanchardi* remained numerically dominant on levels two and three, respectively. One notable change was that chironomids became more abundant than mayflies in summer. Another was that the mayfly *P. guttata*, the dominant invertebrate in spring, was replaced by the mayflies *S. femoratum*, *Centropilum* sp., and *Choroterpes* sp. in summer.

Discussion

Sunfish did not produce an obvious "top-down" trophic effect before or after stream intermittence in 1989. We had predicted that fish would affect lower trophic levels only after stream intermittence when surface exchange (i.e. drift) into fish enclosures was presumably restricted. Overall, fish effects on macroinvertebrates and on lower, noncontiguous trophic levels were not observed. Moreover, fish did not influence the general organization of the food web structure in the enclosures.

In 1990, we tested the hypothesis that fish effects were more likely to occur on bedrock than on coarse substrata. We reasoned that reaches with coarse substrata would provide more refuge space for macroinvertebrate prey, thus decreasing predation intensity, than those on bedrock. Indeed, fish affected macroinvertebrates on bedrock more so than on coarse substrata. This finding agrees with several other studies that report that stream fishes can affect macroinvertebrate densities or assemblage on fine or bedrock substrates in runs or pools (Hemphill and Cooper 1984; Gilliam et al. 1989; Reice 1991). Densities of two taxa on bedrock substratum were significantly affected by fish presence. The detritivorous mayfly *Leptophle-*

TABLE 5. Mean (± 1 SE) absolute densities (ind./m²) of those benthic macroinvertebrates with densities ≥ 1 ind./m² on bedrock and coarse substrates in fish and fishless treatments in July 1990. Macroinvertebrate functional feeding groups, based largely on Merritt and Cummins (1984), are collector/gatherers (CG), shredding detritivores (SD), scrapers (S), herbivore/scavengers (HS), engulfers (E), and piercer/carnivores (PC). * $P < 0.05$, NS = not significant (paired sample t-tests).

Taxon	Functional feeding group	Fish	No fish
Bedrock			
Chironomids (Diptera)		644 $\pm$ 176	406 $\pm$ 48 NS
<i>Stenonema femoratum</i> (Ephemeroptera)	CG	64 $\pm$ 15	128 $\pm$ 42 NS
<i>Centroptilum</i> sp. (Ephemeroptera)	CG	92 $\pm$ 37	82 $\pm$ 36 NS
<i>Callibaetis</i> sp. (Ephemeroptera)	CG	75 $\pm$ 32	31 $\pm$ 13 NS
<i>Choroterpes</i> sp. (Ephemeroptera)	S, CG	18 $\pm$ 6	30 $\pm$ 6 NS
<i>Hydroporus blanchardi</i> Larvae (Coleoptera)	PC	68 $\pm$ 40	21 $\pm$ 14*
<i>Palmaricoria buenoi</i> (Hemiptera)	PC	32 $\pm$ 18	26 $\pm$ 12 NS
<i>Lirceus fontinalis</i> (Isopoda)	SD	10 $\pm$ 4	21 $\pm$ 9 NS
<i>Dannella litta</i> (Ephemeroptera)	CG	22 $\pm$ 11	18 $\pm$ 8 NS
<i>Leptophlebia</i> sp. (Ephemeroptera)	CG	2 $\pm$ 1	26 $\pm$ 13*
<i>Lanthus parvulus</i> (Odonata)	E	9 $\pm$ 4	3 $\pm$ 2 NS
<i>Perithemus</i> sp. (Odonata)	E	2 $\pm$ 1	6 $\pm$ 3 NS
<i>Sialis</i> sp. (Megaloptera)	E	4 $\pm$ 2	1 $\pm$ 1 NS
Coarse substrates			
Chironomids (Diptera)		577 $\pm$ 102	541 $\pm$ 69 NS
<i>Stenonema femoratum</i> (Ephemeroptera)	CG	178 $\pm$ 62	150 $\pm$ 35 NS
<i>Centroptilum</i> sp. (Ephemeroptera)	CG	131 $\pm$ 37	86 $\pm$ 17 NS
<i>Choroterpes</i> sp. (Ephemeroptera)	CG	51 $\pm$ 19	43 $\pm$ 12 NS
<i>Lirceus fontinalis</i> (Isopoda)	SD	33 $\pm$ 9	35 $\pm$ 14 NS
<i>Utaperla</i> sp. (Plecoptera)	CG	12 $\pm$ 5	5 $\pm$ 2 NS
<i>Hydroporus blanchardi</i> larvae (Coleoptera)	PC	14 $\pm$ 4	3 $\pm$ 2 NS
<i>Hydroporus blanchardi</i> adults (Coleoptera)	C	4 $\pm$ 2	2 $\pm$ 2 NS
<i>Leptophlebia</i> sp. (Ephemeroptera)	CG	14 $\pm$ 8	6 $\pm$ 3 NS
<i>Palmaricoria buenoi</i> (Hemiptera)	PC	11 $\pm$ 4	7 $\pm$ 4 NS
<i>Chrysops</i> sp. (Diptera)	CG	8 $\pm$ 6	4 $\pm$ 2 NS
<i>Psephenus herricki</i> (Coleoptera)	S	5 $\pm$ 3	7 $\pm$ 5 NS
<i>Polycentropus</i> sp. (Trichoptera)	E, CG	4 $\pm$ 2	7 $\pm$ 3 NS
<i>Hexatoma</i> sp. (Diptera)	E	6 $\pm$ 2	5 $\pm$ 2 NS
<i>Orconectes rusticus</i> (Decapoda)	HS	4 $\pm$ 2	5 $\pm$ 4 NS

TABLE 6. Mean (± 1 SE) densities (ind./m²) of macroinvertebrates in different microhabitats on bedrock and coarse substrata. Numbers in parentheses are the number of enclosures from which the microhabitat was sampled (nd = no data collected). ** $P < 0.01$, NS = not significant (ANOVA).

Fish presence	<i>Cymbella</i>	Silt/sand	Gravel	Pebble/cobble	Leaf litter
Bedrock					
Fish	881 $\pm$ 209 (7)	914 $\pm$ 72 (2)	1309 $\pm$ 169 (6)	nd	1940 $\pm$ 795 NS (5)
No fish	591 $\pm$ 142 (8)	1060 $\pm$ 0 (1)	1065 $\pm$ 194 (5)	nd	1771 $\pm$ 213** (5)
Coarse substrata					
Fish	nd	766 $\pm$ 205 (5)	1148 $\pm$ 255 (8)	992 $\pm$ 154 (6)	1299 $\pm$ 277 NS (7)
No fish	nd	958 $\pm$ 278 (4)	893 $\pm$ 131 (9)	883 $\pm$ 174 (6)	1219 $\pm$ 203 NS (7)

bia sp. was more abundant in fishless enclosures, while the predaceous beetle *H. blanchardi* was more abundant in enclosures containing fish. The latter resulted in a difference in the proportions of functional feeding groups among fish treatments.

Fish presence, however, did not influence macroinvertebrate densities within microhabitats, regardless of substratum. Dense growths of the stalked diatom *Cymbella* covered microhabitats, particularly over bedrock surfaces. *Cymbella* may have provided enough structural complexity on bedrock surfaces to reduce predation intensity by fish both before and after its senescence (late June), thereby reducing fish effects. Many

empirical studies have shown that the structural complexity of the habitat deters predation and facilitates prey coexistence (e.g. Fraser and Cerri 1982; Lubchenco 1983). Also, macroinvertebrate densities were generally higher in leaf litter than elsewhere, suggesting that leafpacks provide a good refuge from predators and/or are good forage.

Fish density in our fish treatments was 0.1/m², which corresponds to natural fish densities in Hart's Run. This density is low relative to other streams (Gilliam et al. 1989; Reice 1991) and may be too low to significantly affect benthic invertebrate densities throughout the stream. Mean invertebrate densities in

TABLE 7. Mean (± 1 SE) absolute densities (10^3 cells/cm²) of the common diatom taxa on bedrock and coarse substrata in fish and fishless enclosures. NS = not significant at $\alpha = 0.05$ (paired sample t-tests).

Taxon	Fish	No fish
Bedrock		
<i>Achnanthes minutissima</i>	24.2 $\pm$ 4.6	27.5 $\pm$ 6.1 NS
<i>Cymbella cistula</i>	17.2 $\pm$ 4.2	45.1 $\pm$ 11.4 NS
<i>Cymbella minuta</i>	32.7 $\pm$ 12.9	16.3 $\pm$ 3.8 NS
<i>Synedra tenera</i>	4.3 $\pm$ 1.5	5.4 $\pm$ 2.8 NS
Coarse substrata		
<i>Achnanthes minutissima</i>	76.1 $\pm$ 21.4	108.8 $\pm$ 27.9 NS
<i>Cymbella cistula</i>	26.1 $\pm$ 11.5	73.2 $\pm$ 36.8 NS
<i>Cymbella minuta</i>	8.5 $\pm$ 2.5	20.9 $\pm$ 10.3 NS
<i>Synedra tenera</i>	22.9 $\pm$ 11.1	22.7 $\pm$ 6.9 NS

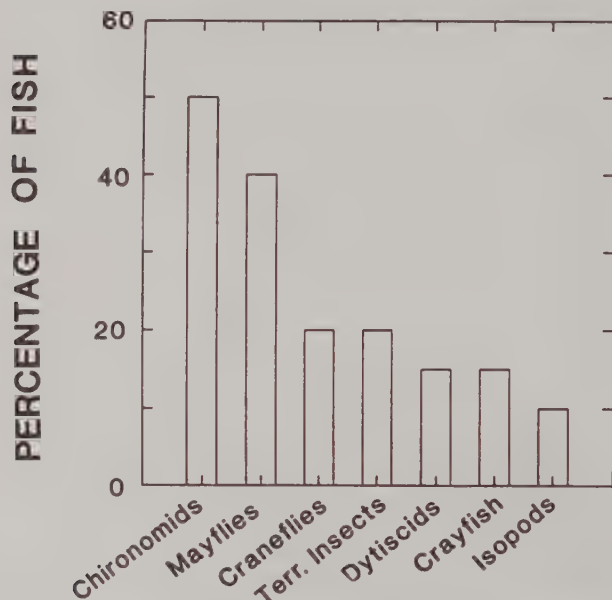


FIG. 1. Proportion of fish with major prey items. Stomachs were flushed in late June and early July. $n = 20$ fish with stomach contents.

both experiments (years) ranged from about 400 to 1100/m²; thus the total number of invertebrates per enclosure half ranged from 8000 to 22 000. Sunfish were enclosed for ≈ 20 d after intermittence in 1989 and a total of 28–39 d in 1990. If one fish eats 50 prey per day, which is not an unreasonable estimate (W. Minckley, pers. comm.), then both enclosed fish could potentially reduce the total number of invertebrates by about 9–25% over these durations. Macroinvertebrate densities were 7.9% lower in enclosures with fish than without in 1989. This result suggests that fish had a modest effect on benthic densities after stream intermittence. Perhaps surface migration was not restricted long enough for pronounced fish effects to be detected. However, other studies have reported predator effects on lower trophic levels over similar durations (Power et al. 1988; Schlosser and Ebel 1989; Power 1990).

Many factors may neutralize the effects of predators on food webs in patchy environments. First, we believe that unobstructed recruitment of invertebrates into fish enclosures was important in minimizing fish effects in both our experiments (years). Natural immigration of prey can probably mask local fish effects even in low-flow conditions (Flecker 1984; Culp

1986; Reice and Edwards 1986), such as those that exist after intermittence. We designed our enclosures to allow for unimpeded drift from upstream areas, recruitment from deeper substrata, and oviposition from aerially flying adult insects. Cooper et al. (1990) stressed that restricting sources of natural recruitment enhances the probability of demonstrating fish effects on lower trophic levels. Their literature survey on predation experiments in streams revealed a negative correlation between cage mesh size and the magnitude of predator impact. A common mesh size used in predation/enclosure experiments is 3.0 mm. We believe that the 6.3-mm mesh of our enclosures was large enough to allow immigration of prey but not too large to allow entry of other fish predators. Careful electroshocking surveys showed that darters were rare in downstream enclosures and absent in upstream enclosures (i.e. the 14 enclosures >500 m upstream). Although small numbers of creek chub (*Semotilus atromaculatus*) fry were occasionally found in enclosures, fry were usually schooled in areas away from enclosures. Additionally, our enclosures did not hinder stream flow. We observed no habitat modifications such as detrital accumulation inside enclosures, a common artifact of field enclosure experiments (Peckarsky and Penton 1990).

Second, flooding and intermittency can clearly influence the population and community patterns of stream organisms (Poff and Ward 1989). In Harts Run, the physical stress of scouring floods in spring and of stream drying in early summer may be more important than fish predation in regulating the food web structure. When scouring floods subside by late spring (mid-May), *Cymbella* grows rapidly. This algal proliferation seems to have little effect on grazing insects because we detected no increase in their densities. Low discharge by early June and intermittency by mid-June restrict fish presence and movements to deeper and more persistent downstream reaches of the stream. Stronger fish effects would be expected in these areas.

Other factors influencing trophic control in Hart's Run probably include primary productivity and fish composition and density. Nutrients in Hart's Run limit algal productivity (Stevenson et al. 1991). Evidence suggests that low primary productivity due to nutrient limitation can negatively affect grazer densities in streams (Stewart 1987; Hart and Robinson 1990). Thus, nutrient limitations may contribute to a "bottom-up" rather than "top-down" pattern of trophic control. In the highly productive Eel River system, Power (1990) found that predatory fish can exert "top-down" trophic effects on grazing fishes and chironomids, which in turn affect algal biomass. Herbivorous fish can directly exert "top-down" effects in productive systems by significantly cropping algal biomass (Power and Matthews 1983; Power et al. 1988). Hart's Run does not contain algivorous fishes, nor does it contain high densities of benthic-feeding fishes. The complete or virtual absence of these kinds of fishes probably also contributes to the modest fish effects observed in this stream.

As noted by Hart and Robinson (1990), our understanding of mechanisms controlling community dynamics of streams is still in its infancy. A clearer understanding of how fish affect trophic and community structure in streams will not be possible until more manipulative studies are conducted that analyze an array of systems and potential abiotic and biotic regulatory factors.

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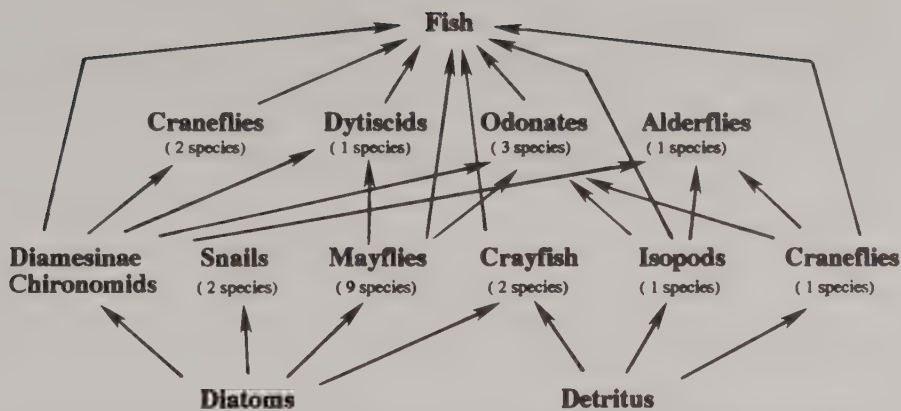


FIG. 2. Generalized food web in enclosures containing fish in runs of Hart's Run in spring and summer. The first three trophic levels are similar in fishless enclosures.

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References

- ALLAN, J. D. 1982. The effects of reduction in trout density on the invertebrate community of a mountain stream. *Ecology* 63: 1444-1455.
- APHA. 1985. Standard methods for the examination of water and wastewater. 16th ed. American Public Health Association, Washington, DC.
- ARSUFFI, T. L., AND K. SUBERKROPP. 1985. Selective feeding by stream caddisfly (Trichoptera) detritivores on leaves with fungal-colonized patches. *Oikos* 45: 50-58.
- BOWLBY, J. N., AND J. C. ROFF. 1986. Trophic structure in southern Ontario streams. *Ecology* 67: 1670-1679.
- CLAY, W. M. 1962. A field manual of Kentucky fishes. Kentucky Department of Fish and Wildlife Resources, Frankfort, KY.
- COOPER, S. D., S. J. WALDE, AND B. L. PECKARSKY. 1990. Prey exchange rates and the impact of predators on prey populations in streams. *Ecology* 71: 1503-1514.
- CULP, J. M. 1986. Experimental evidence that stream macroinvertebrate community structure is unaffected by different densities of coho salmon fry. *J. N. Am. Benthol. Soc.* 5: 140-149.
- CUMMINS, K. W. 1962. An evaluation of some techniques for the collection and analysis of benthic samples with a special emphasis on lotic water. *Am. Midl. Nat.* 67: 477-504.
- FEMINELLA, J. W., M. E. POWER, AND V. H. RESH. 1989. Periphyton responses to invertebrate grazing and riparian canopy in three northern California coastal streams. *Freshwater Biol.* 22: 445-457.
- FLECKER, A. S. 1984. The effects of predation and detritus on the structure of a stream insect community: a field test. *Oecologia (Berl.)* 64: 300-305.
- FLECKER, A. S., AND J. D. ALLAN. 1984. The importance of predation, substrate and spatial refuge in determining lotic insect distributions. *Oecologia (Berl.)* 64: 306-313.
- FRASER, D. F., AND R. D. CERRI. 1982. Experimental evaluation of predator-prey relationships in a patchy environment: consequences for habitat use patterns in minnows. *Ecology* 63: 307-313.
- GILLIAM, J. F., D. F. FRASER, AND A. M. SABAT. 1989. Strong effects of foraging minnows on a stream benthic invertebrate community. *Ecology* 70: 445-452.
- GRIFFITHS, R. W. 1981. The effect of trout predation on the abundance and production of stream insects. Masters thesis, University of British Columbia, Vancouver, B.C.
- HART, D. D., AND C. T. ROBINSON. 1990. Resource limitation in a stream community: phosphorus enrichment effects on periphyton and grazers. *Ecology* 71: 1494-1502.
- HEMPHILL, N., AND S. D. COOPER. 1984. Differences in the community structure of stream pools containing or lacking fish. *Verh. Int. Ver. Limnol.* 22: 1858-1861.
- HILL, W. R., AND B. C. HARVEY. 1990. Periphyton responses to higher trophic levels and light in a shaded stream. *Can. J. Fish. Aquat. Sci.* 47: 2307-2314.
- HOLOMUZKI, J. R., AND J. D. HOYLE. 1990. Effect of predatory fish presence on habitat use and diel movement of the stream amphipod, *Gammarus minus*. *Freshwater Biol.* 24: 509-517.
- HOLOMUZKI, J. R., AND T. M. SHORT. 1988. Habitat use and fish avoidance behaviors by the stream-dwelling isopod *Lirceus fontinalis*. *Oikos* 52: 79-86.
1990. Ontogenetic shifts in habitat use and activity in a stream-dwelling isopod. *Holarct. Ecol.* 13: 300-307.
- LIND, O. T. 1974. Handbook of common methods in limnology. C.V. Mosby Co., St. Louis, MO.
- LUBCHENCO, J. 1983. *Littorina* and *Fucus*: effects of herbivores, substratum heterogeneity and plant escapes during succession. *Ecology* 64: 1116-1123.
- MERRITT, R. W., AND K. W. CUMMINS. 1984. An introduction to the aquatic insects. 2nd ed. Kendall/Hunt Publishing Co., Dubuque, IA.
- MINCKLEY, W. L. 1963. The ecology of a spring stream, Doe Run, Meade County, Kentucky. *Wildl. Monogr.* 11: 1-124.
- PECKARSKY, B. L., AND M. A. PENTON. 1990. Effects of enclosures on stream microhabitat and invertebrate community structure. *J. N. Am. Benthol. Soc.* 9: 249-261.
- POFF, N. L., AND J. V. WARD. 1989. Implications of streamflow variability and predictability for lotic community structure: a regional analysis of stream-flow patterns. *Can. J. Fish. Aquat. Sci.* 46: 1805-1818.
- POWER, M. E. 1990. Effects of fish in a river food web. *Science (Wash., DC)* 250: 811-814.
- POWER, M. E., AND W. J. MATTHEWS. 1983. Algae-grazing minnows (*Camptostoma anomalum*), piscivorous bass (*Micropterus* spp.) and the distribution of attached algae in a small prairie margin stream. *Oecologia (Berl.)* 60: 328-332.
- POWER, M. E., A. V. J. MATTHEWS, AND A. J. STEWART. 1988. Grazer control of algae in an Ozark Mountain stream: effects of short-term exclusion. *Ecology* 69: 1894-1898.
- REICE, S. R. 1991. Effects of detritus loading and fish predation on leafpack breakdown and benthic macroinvertebrates in a woodland stream. *J. N. Am. Benthol. Soc.* 10: 42-56.
- REICE, S. R., AND R. L. EDWARDS. 1986. The effect of vertebrate predation on lotic macroinvertebrate communities in Quebec, Canada. *Can. J. Zool.* 64: 1930-1936.
- RESH, V. H., A. V. BROWN, A. P. COVICH, M. E. GURTZ, S. W. LI, G. W. MINSHALL, S. R. REICE, A. L. SHELDON, J. B. WALLACE, AND R. C. WISSMAR. 1988. The role of disturbance in stream ecology. *J. N. Am. Benthol. Soc.* 7: 433-455.
- ROBINSON, C. T., AND S. R. RUSHFORTH. 1987. Effects of physical disturbance and canopy cover on attached diatom community structure in an Idaho stream. *Hydrobiologia* 154: 49-59.
- SCHLOSSER, I. J., AND K. K. EBEL. 1989. Effects of flow regime and cyprinid predation on a headwater stream. *Ecol. Monogr.* 59: 41-57.
- SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. 2nd ed. W. H. Freeman and Co., San Francisco, CA.
- STEVENSON, R. J. 1984a. How currents on different sides of substrates in streams affect mechanisms of benthic algal accumulation. *Int. Rev. Gesamten Hydrobiol.* 69: 241-262.

- 1984b. Procedures for mounting algae in a syrup medium. Trans. Am. Microsc. Soc. 103: 320–321.
- STEVENSON, R. J., C. G. PETERSEN, D. B. KIRSCHTEL, C. C. KING, AND N. C. TUCHMAN. 1991. Density-dependent growth, ecological strategies, and effects of nutrients and shading on benthic diatom succession in streams. J. Phycol. 27: 59–69.
- STEWART, A. J. 1987. Responses of stream algae to grazing minnows and nutrients: a field test for interactions. Oecologia (Berl.) 72: 17.
- WALDE, S. J., AND R. W. DAVIES. 1984. Invertebrate predation and lotic prey communities: evaluation of in situ enclosure/exclosure experiments. Ecology 65: 1206–1213.
- ZERBA, K. E. 1989. Individual variation in diet of larval tiger salamanders (*Ambystoma tigrinum nebulosum*) in Arizona. Ph.D. thesis, Arizona State University, Tempe, AZ.

Comparison of Chlorophyll *a* Extractions with Ethanol and Dimethyl Sulfoxide/Acetone, and a Concern about Spectrophotometric Phaeopigment Correction

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Chlorophyll *a* (Chl *a*) in water samples from three mesotrophic to eutrophic lakes in north-central Alberta was extracted with one of three solvents (95% ethanol, 90% ethanol, or a 2:3 mixture of dimethyl sulfoxide and 90% acetone (DMSO/acetone)) and analyzed by two techniques (spectrophotometry and high pressure liquid chromatography (HPLC)). The dominant phytoplankton were blue-green algae and diatoms. Total Chl *a* concentrations (i.e. no correction for phaeopigments (Pha)) were not significantly different among solvents ($P > 0.5$). Total Chl *a* concentrations from spectrophotometric analyses were significantly higher than those from HPLC analyses (4.2 ± 0.88 and $2.6 \pm 0.50 \mu\text{g}\cdot\text{L}^{-1}$, respectively, $P < 0.05$). Pha concentrations derived by spectrophotometry were 64 times higher than those derived by HPLC (1.7 ± 0.52 and $0.025 \pm 0.01 \mu\text{g}\cdot\text{L}^{-1}$, respectively, $P < 0.005$). Thus, spectrophotometry appears to dramatically overestimate Pha concentrations and may overestimate total Chl *a* (i.e. no correction for Pha). Therefore, ethanol and DMSO/acetone are equally suitable for Chl *a* extraction from natural populations dominated by blue-green algae and/or diatoms, but if information on Pha and/or accessory pigments is required, HPLC analyses are the appropriate route rather than spectrophotometry.

La chlorophylle *a* (Chl *a*) présente dans les échantillons d'eau provenant de trois lacs mésotrophes-eutrophes de la région centre-nord de l'Alberta a été extraite à l'aide de l'un des trois solvants suivants, éthanol 95 %, éthanol 90 % ou un mélange 2 : 3 de diméthylsulfoxyde et d'acétone 90 % (DMSO/acétone) et a été dosée par deux techniques (spectrophotométrie et chromatographie liquide haute pression (CLHP)). Les éléments dominants du phytoplancton étaient les algues bleues et les diatomées. Les différences entre les concentrations de Chl *a* totale (c'est-à-dire sans correction pour la concentration de phaeopigment (Pha)) obtenues avec chacun des solvants n'étaient pas significatives ($P > 0,5$). Les concentrations de Chl *a* totale obtenues par dosage spectrophotométrique étaient significativement plus élevées que celles obtenues par CLHP (respectivement $4,2 \pm 0,88$ et $2,6 \pm 0,50 \mu\text{g}\cdot\text{L}^{-1}$, $P < 0,05$). Les concentrations de Pha obtenues par dosage spectrophotométrique étaient 64 fois plus élevées que celles obtenues par CLHP (respectivement $1,7 \pm 0,52$ et $0,025 \pm 0,01 \mu\text{g}\cdot\text{L}^{-1}$, $P < 0,005$). Ainsi, la spectrophotométrie semble surestimer de manière considérable les concentrations de Pha et peut surestimer la concentration de Chl *a* totale (c'est-à-dire sans correction pour Pha). Par conséquent, l'éthanol et le mélange DMSO/acétone semblent convenir tout aussi bien pour l'extraction de la Chl *a* à partir de populations naturelles dominées par les algues bleues ou les diatomées; toutefois, si l'on veut obtenir des données sur la concentration de Pha ou des pigments accessoires, le dosage par CHLP est préférable à la spectrophotométrie.

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The accuracy of chlorophyll *a* (Chl *a*) measurements (and subsequent estimates of algal biomass) depends on the extraction efficiency of the solvent used and the species of phytoplankton present, as well as the specificity of the technique used for analysis. Numerous organic solvents have been used to extract Chl *a* from algae; however, acetone is the most common (Richards and Thompson 1952; Moss 1967; Strickland and Parsons 1972; Daemen 1986), particularly for marine phytoplankton. Unfortunately, the extraction efficiency of acetone is low for most green and some blue-green algae, particularly freshwater species; methanol, ethanol, and various combinations of dimethyl sulfoxide and 90% acetone (DMSO/acetone) are superior (Shoaf and Lium 1976; Holm-Hansen and Riemann 1978; Stauffer et al. 1979; Burnison 1980; Sartory and Grobbelaar 1984). Methanol and ethanol have similar

extraction efficiencies from cultures of *Scenedesmus quadricauda*, *Microcystis aeruginosa*, *Chlorella vulgaris* (in exponential phase), *Selenastrum capricornutum*, and *Pediastrum duplex*, as well as from natural samples dominated by *Microcystis aeruginosa* and by diatoms (Sartory and Grobbelaar 1984; Jespersen and Christoffersen 1987).

Various techniques for Chl *a* analysis have been compared, including spectrophotometry, fluorometry, spectrofluorometry, and high pressure liquid chromatography (HPLC). Recent studies have reported that traditional nonseparative spectrophotometric and fluorometric techniques yield higher Chl *a* values than HPLC due to their erroneous measurement (and inclusion) of pigments and degradation products other than Chl *a* (Mantoura and Llewellyn 1983; Sartory 1985; Daemen 1986; Murray et al. 1986; Neveux et al. 1990). Neveux et al. (1990) also found major discrepancies in phaeopigments (Pha) among different techniques, with spectrophotometry and fluorometry producing the highest concentrations, HPLC producing the

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lowest, and spectrofluorometry producing intermediate concentrations.

The primary goal of our study was to compare Chl *a* extracted from natural assemblages of freshwater blue-green algae and diatoms with ethanol (either 95 or 90%) and a 2:3 mixture of DMSO and 90% acetone (DMSO/acetone). Comparisons of the extraction efficiencies of ethanol and DMSO/acetone for blue-green algae and/or diatoms have not been published, but heated 90% ethanol and heated DMSO/acetone extract similar amounts of total Chl *a* (Chl *a* + Pha) from cultures of a green alga, *Selenastrum capricornutum* (Sartory and Grobbelaar 1984). A secondary goal was to observe the relative accuracy of Chl *a* and Pha concentrations derived from spectrophotometric and HPLC analyses for natural assemblages of freshwater blue-green algae and diatoms. These values were then compared with Chl *a* and Pha determined with these techniques from algal cultures and natural assemblages of marine and estuarine algae (Mantoura and Llewellyn 1983; Sartory 1985; Daemen 1986; Murray et al. 1986; Neveux et al. 1990).

Methods and Materials

Two mesotrophic lakes (Narrow and Long) and one naturally eutrophic lake (Island) in north-central Alberta, dominated by blue-green algae (*Aphanizomenon flos-aquae* and *Anabaena* spp.) and diatoms (*Asterionella formosa* and *Cyclotella* sp.), were sampled four to eight times during the summer of 1986. Water collected with an aluminum drop-sleeve water bottle from two to 10 depths in the euphotic zone was stored in 2-L brown polyethylene bottles in coolers with ice packs until they could be returned to the laboratory and refrigerated at 4°C.

Four 300-mL (Island Lake) or four 700-mL (Narrow and Long lakes) subsamples were filtered within 8 h of collection onto Whatman GF/C filters at -50 kPa. (Subsamples from the eutrophic and mesotrophic lakes were of different volumes to prevent clogging of the filter pores during filtration.) The two filters for 95% ethanol extraction were folded and placed in plastic petri dishes with tight-fitting lids. The two filters for DMSO/acetone extraction were folded and placed in 20-mL glass vials with teflon-lined screw caps. The vials and petri dishes were wrapped in foil and stored at -20°C for up to 3 wk before spectrophotometric analysis and for several months before HPLC analysis. All work was performed under low light conditions.

Similar sample collections were made on one date in July 1989, except that a greater volume of water was collected from each site at each depth and pooled. From each pooled sample, four filters were prepared for 95% ethanol extraction, five for DMSO/acetone extraction, and six for 90% ethanol extraction. Four filters from each solvent were analyzed spectrophotometrically and the remainder were analyzed by HPLC. Subsamples were the same volumes as in 1986 for each lake. All filters were stored in plastic petri dishes with tight-fitting lids, except those which were to be extracted with DMSO/acetone and analyzed by spectrophotometry, which were stored in the same type of glass vials used in 1986.

Two spectrophotometric methods were evaluated in 1986 (extraction with 95% ethanol and DMSO/acetone). Extraction with 95% ethanol was based on M. Ostrofsky's (Biology Department, Alleghany College, Meadville, PA, unpubl.) method described in Bergmann and Peters (1980), which measures total Chl *a* only. MgCO_3 was added as the last 25 mL of subsample was filtered to prevent degradation of Chl *a* in the extract (Vollenweider 1974). DMSO/acetone extraction was

based on Burnison (1980), which includes correction for Pha by acidification of the sample and which does not include the addition of MgCO_3 . The filter/solvent slurries (from both solvents) were stored overnight in the refrigerator. Absorbance was read with a Bausch and Lomb Spectronic 100 spectrophotometer (8-mL cell, 10-cm path length, 8.0-nm slit width) at 750, 665, and 649 nm against a 95% ethanol blank and at 750 and 664 nm against a DMSO/acetone blank. The spectrophotometer's wavelength settings were calibrated with a pure Chl *a* standard (Sigma Chemicals, St. Louis, MO). The instrument was zeroed at each wavelength against the appropriate solvent blank.

In 1989, a third spectrophotometric method was added to the evaluation: extraction with 90% ethanol was based on Nusch (1980), which includes correction for Pha by acidification of the sample. MgCO_3 was not added to the samples. The filter/solvent slurries (from all three solvents) were stored overnight in the refrigerator before absorbance was read with a Milton Roy Spectronic 1001 spectrophotometer (8-mL cell, 10-cm path length, 2.0-nm slit width) at 750 and 665 nm against a 90% ethanol blank. This instrument was calibrated to the Spectronic 100 used in 1986, and the appropriate correction factor was applied to the 1989 concentrations.

In 1989, samples for HPLC analysis were prepared as for spectrophotometric determination (see above). One frozen filter from each sample was extracted with DMSO/acetone according to Burnison (1980) and two filters from each sample were extracted with 90% ethanol according to Sartory and Grobbelaar (1984). All samples were allowed to cool at room temperature in the dark for 1 h. One millilitre of the extract was centrifuged at 4000g in a Fisher centrifuge (model 59) for 1 min to quickly remove the glass fibers. The supernatant was filtered through a 4-mm PTFE filter (0.2- μm pore size) before 100 μL was injected onto the column. Clarified samples were stored in the dark at room temperature for no longer than 30 min before injection. The HPLC analysis was performed with a Waters HPLC system with the 600E multisolvent delivery system and an LC-18 reverse phase column (Supelco, 5- μm particle size). The isocratic mobile phase was methanol, water, and acetone (42:5:53). A Varian 2070 spectrofluorometer (excitation 420 nm, emission 665 nm) was used for pigment quantification (Chl *a* = Chl *a* peak plus allomerized Chl *a* peaks). Purified Chl *a* and Chl *b* were obtained from Sigma Chemicals (catalog Nos. C-6144 and C-5878) and Pha were prepared by acidification (B. K. Burnison, unpubl.). Ninety-percent acetone solutions of each chlorophyll and phaeophytin were prepared and quantified spectrophotometrically with the specific absorption coefficients of Jeffrey and Humphrey (1975).

Total Chl *a* (no correction for Pha) concentrations derived by spectrophotometry from 95% ethanol, 90% ethanol, and DMSO/acetone are referred to as TS_{95} , TS_{90} , and TS_{DA} , respectively. Chl *a* concentrations (corrected for phaeopigments) from 90% ethanol and DMSO/acetone and measured by spectrophotometry are referred to as CS_{90} and CS_{DA} , respectively, while corresponding Pha concentrations are referred to as PS_{90} and PS_{DA} , respectively. Chl *a* concentrations from HPLC analysis of 90% ethanol and DMSO/acetone extracts are referred to as CH_{90} and CH_{DA} , respectively, while corresponding Pha concentrations are referred to as PH_{90} and PH_{DA} , respectively. Chl *a* and Pha measured by HPLC were combined to provide "total" Chl *a* concentrations for comparison with total Chl *a* estimates determined by spectrophotometry and are referred to as TH_{90} and TH_{DA} (from 90% ethanol and DMSO/acetone extracts, respectively).

Statistical analyses were performed on the mean concentrations (when there was more than one subsample analyzed) from the various solvents and techniques for each sample with the Statistical Analysis System (SAS). Analyses of variance (ANOVA) were performed to determine differences in Chl *a* among solvents (95% ethanol, 90% ethanol, and DMSO/acetone) and/or between techniques (spectrophotometry and HPLC). The Student–Newman–Keuls (SNK) test was used for multiple comparisons of total Chl *a* among the three solvents.

Results

Chl *a*, Pha, and total Chl *a* concentrations from the two techniques and three solvents are summarized in Table 1. A composite HPLC chromatogram of chlorophylls *a* and *b* and phaeophytins *a* and *b* is shown in Fig. 1A, and the HPLC chromatogram of the DMSO/acetone and 90% ethanol extracts of one of the samples is presented in Fig. 1B.

All total Chl *a* concentrations (TS₉₅, TS₉₀, TS_{DA}, TH₉₀, and TH_{DA}) from both years were compared by two-factor ANOVA (technique × solvent). The three solvents did not yield significantly different total Chl *a* concentrations ($F = 0.35$, $df = 2, 190$, $P > 0.5$), nor was there significant interaction between techniques and solvents ($F = 0.38$, $df = 1, 190$, $P > 0.5$). Multiple comparisons also revealed no significant difference between total Chl *a* concentrations from the three solvents (SNK, $df = 190$, $P > 0.05$). The three solvents (95% ethanol, 90% ethanol, and DMSO/acetone) are thus comparable for Chl *a* extraction from natural assemblages of freshwater blue-green algae and diatoms. The two techniques yielded significantly different total Chl *a* concentrations ($F = 6.71$, $df = 1, 190$, $P < 0.05$). However, HPLC analysis was not done in 1986, so only 1989 data were used for further comparisons of techniques.

TS₉₀, TS_{DA}, TH₉₀, and TH_{DA} concentrations from 1989 were compared by blocked two-factor ANOVA to determine if the techniques yielded different concentrations. (TS₉₅ data were not included, since 95% ethanol extracts were only analyzed by spectrophotometry.) Mean total Chl *a* concentrations from spectrophotometry and HPLC (from both solvents combined) were 4.2 and 2.6 $\mu\text{g}\cdot\text{L}^{-1}$, respectively, and were significantly different ($F = 8.25$, $df = 1, 30$, $P < 0.05$). As with the comparison of the complete data set, there was no significant difference in total Chl *a* between solvents with this data set ($F = 1.35$, $df = 1, 30$, $P > 0.25$), nor were there significant interactions between techniques and solvents ($F = 0.72$, $df = 1, 30$, $P > 0.25$). Thus, choice of technique had a significant impact on total Chl *a* estimates, with HPLC analysis

producing lower total Chl *a* concentrations than spectrophotometric analysis. Choice of solvent had no significant impact on total Chl *a* estimates.

PS₉₀, PS_{DA}, PH₉₀, and PH_{DA} concentrations from 1989 were compared by blocked two-factor ANOVA (technique × solvent), as were CS₉₀, CS_{DA}, CH₉₀, and CH_{DA} concentrations from 1989, to determine if solvent or technique affected Pha or Chl *a* concentrations. The two techniques yielded significantly different Pha concentrations ($F = 12.92$, $df = 1, 30$, $P < 0.005$), but there was no significant difference in Pha between solvents ($F = 1.67$, $df = 1, 30$, $P > 0.1$), nor was interaction between techniques and solvents significant ($F = 1.73$, $df = 1, 30$, $P > 0.1$). Pha measured by spectrophotometry ranged from 18 to 64% (mean = 30%) of total Chl *a* (mean Pha concentration = 1.7 $\mu\text{g}\cdot\text{L}^{-1}$). Pha contributed only 0–7% (mean = 0.8%) of total Chl *a* measured by HPLC (mean Pha concentration = 0.025 $\mu\text{g}\cdot\text{L}^{-1}$). There was no significant difference in Chl *a* between techniques ($F = 0.19$, $df = 1, 30$, $P > 0.5$) or between solvents ($F = 0.04$, $df = 1, 30$, $P > 0.75$), nor was interaction between techniques and solvents significant ($F = 0.48$, $df = 1, 30$, $P > 0.25$). Results of this part of the study confirm that 90% ethanol and DMSO/acetone are equally suitable for extracting Chl *a* from natural assemblages of freshwater blue-green algae and diatoms. However, spectrophotometry may overestimate Pha and total Chl *a* concentrations.

Discussion

Historically, acetone has been the solvent of choice for Chl *a* extraction (especially in marine systems), but use of ethanol and DMSO is becoming more common for freshwater phytoplankton, where green and blue-green algae (from which extraction with acetone is poor) form a large proportion of the biomass. The 95% ethanol method used in the present study has been used in the Limnology Laboratory at the University of Alberta for over 10 yr for Chl *a* analysis (Prepas and Trew 1983; Trimbee and Prepas 1987; Prepas and Murphy 1988; Prepas et al. 1990), as well as by others (Kalff 1983; Smith et al. 1984; Kalff and Watson 1986; Morin and Peters 1988). Ninety-percent ethanol has also been used for Chl *a* extraction (Nusch 1980; Sartory and Grobbelaar 1984; Sartory 1985; Jespersen and Christoffersen 1987; Jacobsen and Rai 1990). Various studies have also been published in which DMSO (with and without acetone) was used for Chl *a* extraction (Seely et al. 1972; Shoaf and Lium 1976; Hiscox and Israelstam 1979; Burdison 1980; Speziale et al. 1984).

TABLE 1. Mean concentrations of phaeopigment-corrected Chl *a*, Pha, and total Chl *a* (i.e. Chl *a* + Pha) ($\mu\text{g}\cdot\text{L}^{-1}$) from three solvents and two techniques in water samples collected from three mesotrophic to eutrophic Alberta lakes. SE = standard error of mean concentration, Spec = concentrations derived by spectrophotometry, HPLC = concentrations derived by HPLC, $n = 70$ in 1986, and $n = 11$ in 1989. Blanks indicate no data.

Technique	Solvent	Chl <i>a</i>	SE	Pha	SE	Total Chl <i>a</i>	SE
Spec (1986)	95% ethanol					6.3	0.67
	DMSO/acetone	3.8	0.43	3.2	0.57	7.0	0.90
Spec (1989)	95% ethanol					3.2	0.81
	90% ethanol	2.4	0.65	2.3	0.98	4.7	1.56
HPLC (1989)	DMSO/acetone	2.5	0.64	1.1	0.31	3.6	0.90
	90% ethanol	2.6	0.69	0.02	0.008	2.6	0.69
	DMSO/acetone	2.5	0.76	0.03	0.018	2.5	0.77

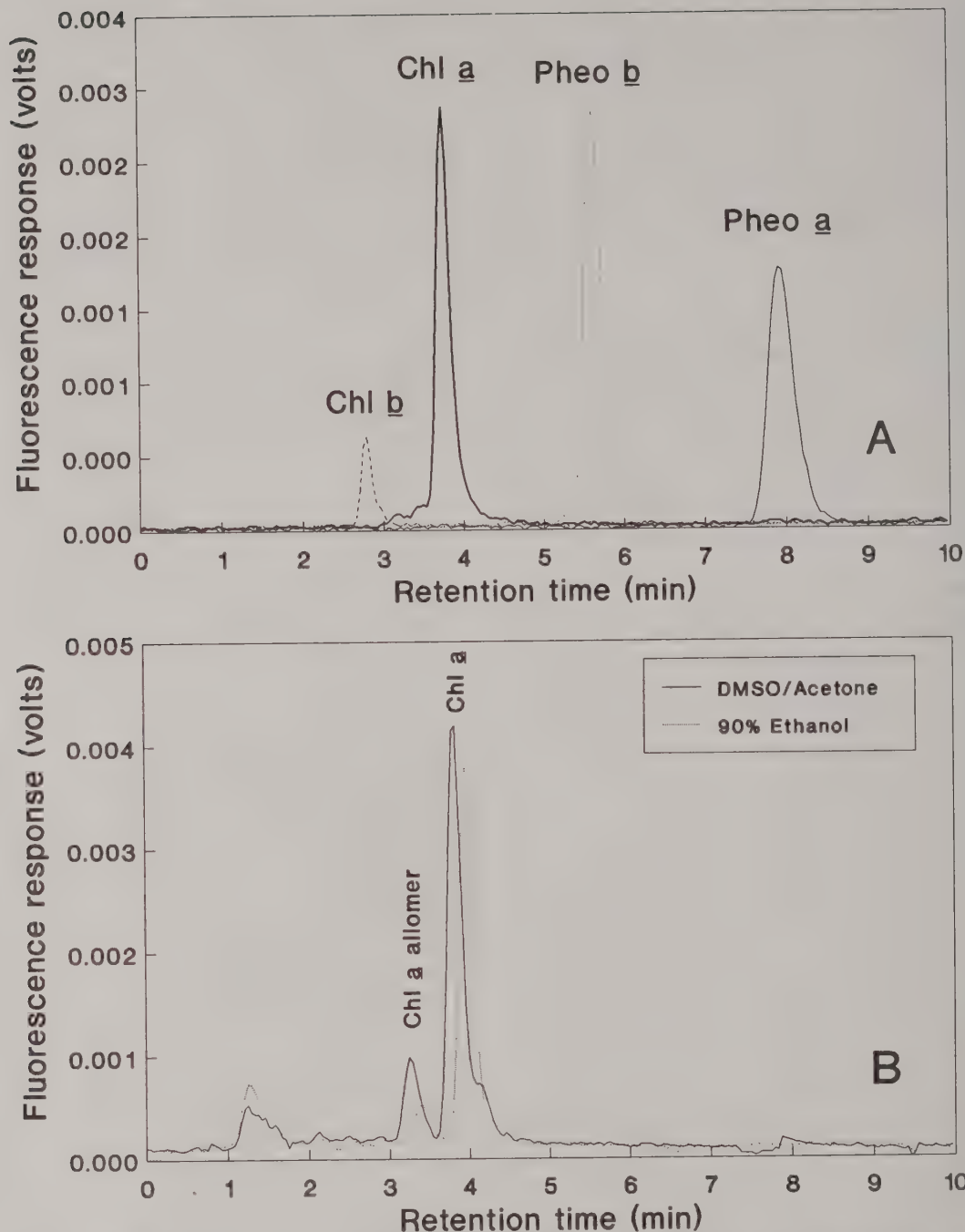


FIG. 1. (A) Composite HPLC chromatogram of chlorophylls *a* and *b* and phaeophytins *a* and *b*. (B) HPLC chromatogram of DMSO/acetone extract (solid curve) and 90% ethanol extract (dotted curve) of samples taken from Island Lake at a depth of 5.5 m.

We recognize that there are several differences (besides solvents) between the spectrophotometric methods compared in this study. However, we wanted to make as few alterations as possible to the published methods. First, MgCO_3 was added to samples extracted with 95% ethanol, but it was not added to those extracted with DMSO/acetone or 90% ethanol. The purpose of MgCO_3 was to prevent degradation of Chl *a* during storage and extraction (Vollenweider 1974). Although filters analyzed by HPLC were stored for considerably longer periods

of time before analysis (several months) than were filters analyzed by spectrophotometry (up to 3 wk), HPLC Pha concentrations were much lower than those derived by spectrophotometry (0.025 and $1.7 \mu\text{g}\cdot\text{L}^{-1}$, respectively). These results are consistent with Sartory and Grobbelaar's (1984) conclusion that the addition of MgCO_3 has no effect on Chl *a* degradation. Furthermore, numerous studies have reported that MgCO_3 adsorbs Pha, thus resulting in underestimates of Pha concentrations (Holm-Hansen and Riemann 1978). Adsorption

of Pha by MgCO_3 may explain why mean TS_{95} concentrations (6.3 and $3.2 \mu\text{g}\cdot\text{L}^{-1}$ in 1986 and 1989, respectively) were slightly lower than mean TS_{DA} concentrations (7.0 and $3.6 \mu\text{g}\cdot\text{L}^{-1}$ in 1986 and 1989, respectively) and mean TS_{90} concentration in 1989 ($4.7 \mu\text{g}\cdot\text{L}^{-1}$). Second, filters prepared for DMSO/acetone extraction followed by spectrophotometric analysis were placed in glass vials with teflon-lined lids and then frozen, while all other filters were placed in plastic petri plates with tight-fitting lids and frozen. However, the similarity among total Chl *a* concentrations, based on the three solvents, indicates that the use of different storage containers did not affect total Chl *a* concentrations.

The lack of significant difference between total Chl *a* concentrations from the various solvents within each technique indicates that the extraction efficiencies of 95% ethanol, 90% ethanol, and DMSO/acetone are comparable for these natural assemblages of predominantly blue-green algae and diatoms. This information complements Sartory and Gobbelaar's (1984) findings that heated ethanol and heated DMSO/acetone are equally efficient for extracting total Chl *a* from *Selenastrum capricornutum*.

Besides extraction efficiency, toxicity and cost may also be considered when selecting solvents for Chl *a* analysis. DMSO absorbs rapidly into the skin (Rammler and Zaffaroni 1967), resulting in irritation, redness, itching, and scaling (Windholz 1976). However, its cutaneous toxicity is relatively low (LD_{50} in rats = $40 \text{ g}\cdot\text{kg}^{-1}$ (Mason 1971)). Ethanol, DMSO, and acetone all have relatively low oral toxicities (LD_{50} in rats = $13.7 \text{ g}\cdot\text{kg}^{-1}$, $>20 \text{ g}\cdot\text{kg}^{-1}$, and $10.7 \text{ mL}\cdot\text{kg}^{-1}$, respectively (Windholz 1976)). The cost of ethanol is about one-fourth that of DMSO/acetone, which becomes important in studies involving analysis of a large number of samples.

Total Chl *a* concentrations determined by HPLC from both ethanol and DMSO/acetone were lower than those derived by spectrophotometry (Table 1). Previous studies have attributed this difference to the presence of accessory chlorophylls (*b* and *c*), other green or blue-green pigments, carotenoid pigments, and/or degradation products that have absorbances similar to Chl *a* but that are chromatographically distinguishable from Chl *a* (Gieskes and Kraay 1983; Mantoura and Llewellyn 1983; Hallegraeff and Jeffrey 1985; Sartory 1985; Daemen 1986; Schanz and Rai 1988; Jacobsen and Rai 1990; Neveux et al. 1990). These compounds interfere with spectrophotometric analysis, resulting in overestimations of total Chl *a*. We made corrections for phaeopigments, but other compounds may have been present in our samples that were not distinguished by spectrophotometry and that were thus included in spectrophotometric Chl *a* measurements. Figure 1A shows the chromatogram of the calibration pigments. The pigments were well separated within the 10-min isocratic run. Chl *b* does not give an equal fluorescent response under the excitation and emission wavelengths chosen. Figure 1B shows a typical chromatogram of a phytoplankton sample. The fluorescence detector is very selective and only the chlorophyllous pigments respond. In this particular sample, DMSO/acetone extracted more pigment from the algae than did 90% ethanol. The slight discrepancy between the Chl *a* peak between the two solvents lies within the ± 0.2 -min window. DMSO/acetone also gave a higher amount of alomerized Chl *a*, but this was added onto the Chl *a* peak (Murray et al. 1986). Fluorescence peaks (retention time about 1.3 min) were noted in both the DMSO/acetone and 90% ethanol extracts and comprised 13.5 and 21.2% of the Chl *a* response, respectively. However, until a thorough analysis of this material is done, it is not known whether it is solely respon-

sible for the difference between HPLC and spectrophotometric analysis of phytoplankton. Certainly, chlorophyllides will be present in this fraction (Murray et al. 1986).

The proportion of phaeopigments was considerably higher in both 90% ethanol and DMSO/acetone extracts analyzed by spectrophotometry (mean = 30%) than in extracts analyzed by HPLC (mean = 0.8%) (see Table 1). Murray et al. (1986) and Neveux et al. (1990) also found discrepancies in phaeopigments between techniques and concluded that spectrophotometric measurement of phaeopigments is often unreliable. If spectrophotometry overestimates Pha concentrations, corresponding Chl *a* concentrations (corrected for Pha) may be similarly underestimated. However, in our study, there were no significant differences in Chl *a* between techniques, even though Pha concentrations were significantly different between techniques. This is likely due to the large proportion of Chl *a* in total Chl *a* concentrations (mean = 99.2 and 70% for HPLC and spectrophotometry, respectively) compared with the smaller proportion of Pha in total Chl *a* concentrations (mean = 0.8 and 30% for HPLC and spectrophotometry, respectively).

We conclude that ethanol is an appropriate solvent for extracting Chl *a* from natural assemblages of freshwater blue-green algae and/or diatoms found in our lakes. Its extraction efficiency and health hazards are similar to DMSO/acetone, but it lacks DMSO's strong, unpleasant odour and it is less expensive. Considering these advantages, we are surprised that it is not used more widely by limnologists for Chl *a* extraction.

Results from our study indicate that spectrophotometry is a suitable technique for determining Chl *a* (corrected for Pha). Samples can be processed rapidly and with relatively little technical skill. Spectrophotometric determination of Pha is not recommended because of its unreliable nature. For studies requiring measurements of Pha or accessory pigments, HPLC analysis is recommended.

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References

- BERGMANN, M., AND R. H. PETERS. 1980. A simple reflectance method for the measurement of particulate pigment in lake water and its application to phosphorus-chlorophyll-seston relationships. *Can. J. Fish. Aquat. Sci.* 37: 111-114.
- BURNISON, B. K. 1980. Modified dimethyl sulfoxide (DMSO) extraction for chlorophyll analysis of phytoplankton. *Can. J. Fish. Aquat. Sci.* 37: 729-733.
- DAEMEN, E. A. M. J. 1986. Comparison of methods for the determination of chlorophyll in estuarine sediments. *Neth. J. Sea Res.* 20: 21-28.
- GIESKES, W. W., AND G. W. KRAAY. 1983. Unknown chlorophyll *a* derivatives in the North Sea and the tropical Atlantic Ocean revealed by HPLC analysis. *Limnol. Oceanogr.* 28: 757-766.
- HALLEGRAEFF, G. M., AND S. W. JEFFREY. 1985. Description of new chlorophyll *a* alteration products in marine phytoplankton. *Deep-Sea Res.* 32: 697-705.

- HISCOX, J. D., AND G. F. ISRAELSTAM. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* 57: 1332-1334.
- HOLM-HANSEN, O., AND B. RIEMANN. 1978. Chlorophyll *a* determination: improvements in methodology. *Oikos* 30: 438-447.
- JACOBSEN, T. R., AND H. RAI. 1990. Comparison of spectrophotometric, fluorometric and high performance liquid chromatography methods for determination of chlorophyll *a* in aquatic samples: effects of solvent and extraction procedures. *Int. Rev. Gesamten Hydrobiol.* 75: 207-217.
- JEFFREY, S. W., AND G. F. HUMPHREY. 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*, *c*₁ and *c*₂ in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz.* 167: 191-194.
- JESPERSEN, A.-M., AND K. CHRISTOFFERSEN. 1987. Measurements of chlorophyll-*a* from phytoplankton using ethanol as extraction solvent. *Arch. Hydrobiol.* 109: 445-454.
- KALFF, J. 1983. Phosphorus limitation in some tropical African lakes. *Hydrobiologia* 100: 101-112.
- KALFF, J., AND S. WATSON. 1986. Phytoplankton and their dynamics in two tropical lakes: a tropical and temperate zone comparison. *Hydrobiologia* 138: 161-176.
- MANTOURA, R. F. C., AND C. A. LLEWELLYN. 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. *Anal. Chim. Acta* 151: 297-314.
- MASON, M. M. 1971. Toxicology of DMSO in animals, p. 113-131. *In* S. W. Jacob, E. E. Rosenbaum, and D. C. Wood [ed.] *Basic concepts of DMSO*. Vol. 1. Marcel Dekker, Inc., New York, NY.
- MORIN, A., AND R. H. PETERS. 1988. Effects of microhabitat features, seston quality, and periphyton abundance of overwintering blackfly larvae in southern Quebec. *Limnol. Oceanogr.* 33: 431-446.
- MOSS, B. 1967. A spectrophotometric method for the estimation of percentage degradation of chlorophylls to pheo-pigments in extracts of algae. *Limnol. Oceanogr.* 12: 335-340.
- MURRAY, A. P., C. F. GIBBS, A. R. LONGMORE, AND D. J. FLETT. 1986. Determination of chlorophyll in marine waters: intercomparison of a rapid HPLC method with full HPLC, spectrophotometric and fluorometric methods. *Mar. Chem.* 19: 211-227.
- NEVEUX, J., D. DELMAS, J. C. ROMANO, P. ALGARRA, L. IGNATIADES, A. HERBLAND, P. MORAND, A. NEORI, D. BONIN, J. BARBE, A. SUKENIK, AND T. BERMAN. 1990. Comparison of chlorophyll and phaeopigment determinations by spectrophotometric, fluorometric, spectrofluorometric and HPLC methods. *Mar. Microb. Food Webs* 4: 217-238.
- NUSCH, E. A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* 14: 14-36.
- PREPAS, E. E., AND T. P. MURPHY. 1988. Sediment-water interactions in farm dugouts previously treated with copper sulfate. *Lake Reservoir Manage.* 4: 161-168.
- PREPAS, E. E., T. P. MURPHY, J. M. CROSBY, D. T. WALTY, J. T. LIM, J. BABIN, AND P. A. CHAMBERS. 1990. Reduction of phosphorus and chlorophyll *a* concentrations following CaCO₃ and Ca(OH)₂ additions to hypereutrophic Figure Eight Lake, Alberta. *Environ. Sci. Technol.* 24: 1252-1258.
- PREPAS, E. E., AND D. O. TREW. 1983. Evaluation of the phosphorus-chlorophyll relationship for lakes off the Precambrian Shield in western Canada. *Can. J. Fish. Aquat. Sci.* 40: 27-35.
- RAMMLER, D. H., AND A. ZAFFARONI. 1967. Biological implications of DMSO based on a review of its chemical properties. *Ann. N.Y. Acad. Sci.* 141: 13-23.
- RICHARDS, F. A., AND T. G. THOMPSON. 1952. The estimation and characterization of plankton populations by pigment analysis. II. A spectrophotometric method for the estimation of plankton pigment. *J. Mar. Res.* 11: 156-172.
- SARTORY, D. P. 1985. The determination of algal chlorophyllous pigments by high performance liquid chromatography and spectrophotometry. *Water. Res.* 19: 605-610.
- SARTORY, D. P., AND J. U. GROBBELAAR. 1984. Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia* 114: 177-187.
- SCHANZ, F., AND H. RAI. 1988. Extract preparation and comparison of fluorometric, chromatographic (HPLC) and spectrophotometric determinations of chlorophyll-*a*. *Arch. Hydrobiol.* 112: 533-539.
- SEELY, G. R., M. J. DUNCAN, AND W. E. VIDAVER. 1972. Preparative and analytical extraction of pigments from brown algae with dimethyl sulfoxide. *Mar. Biol.* 12: 184-188.
- SHOAF, W. T., AND B. W. LIUM. 1976. Improved extraction of chlorophyll *a* and *b* from algae using dimethyl sulfoxide. *Limnol. Oceanogr.* 21: 926-928.
- SMITH, V. H., F. H. RIGLER, O. CHOULIK, M. DIAMOND, S. GRIESBACH, AND D. SKRABA. 1984. Effects of phosphorus fertilization on phytoplankton biomass and phosphorus retention in subarctic Quebec lakes. *Verh. Int. Ver. Limnol.* 22: 376-382.
- SPEZIALE, B. J., S. P. SCHREINER, P. A. GIAMMATTEO, AND J. E. SCHINDLER. 1984. Comparison of *N,N*-dimethylformamide, dimethyl sulfoxide, and acetone for extraction of phytoplankton chlorophyll. *Can. J. Fish. Aquat. Sci.* 41: 1519-1522.
- STAUFFER, R. E., G. F. LEE, AND D. E. ARMSTRONG. 1979. Estimating chlorophyll extraction biases. *J. Fish. Res. Board Can.* 36: 152-157.
- STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis. 2nd ed. *Bull. Fish. Res. Board Can.* 167.
- TRIMBEE, A. M., AND E. E. PREPAS. 1987. Evaluation of total phosphorus as a predictor of the relative biomass of blue-green algae with emphasis on Alberta lakes. *Can. J. Fish. Aquat. Sci.* 44: 1337-1342.
- VOLLENWEIDER, R. A. 1974. A manual on methods for measuring primary production in aquatic environments. IBP Handbook No. 12. 2nd ed. Blackwell, Oxford.
- WINDHOLZ, M. [ED.] 1976. The Merck index. Merck and Co. Inc., Rahway, NJ. 1313 p.

Effects of Sediment Transport on Survival of Salmonid Embryos in a Natural Stream: A Simulation Approach

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A model is presented that simulates the effects of streamflow and sediment transport on survival of salmonid embryos incubating in spawning gravels in a natural channel. Components of the model include a 6-yr streamflow record, an empirical bedload-transport function, a relation between transport and infiltration of sandy bedload into a gravel bed, effects of fine-sediment infiltration on gravel properties, and functions relating embryo survival to gravel properties. High-flow events drive temporal variations in survival; cross-channel variations in bedload transport cause spatial variations. Expected survival, as a result, varies widely from year to year and between spawning runs in a single year. Alternative functions from previous research that relate survival to fine-sediment concentration in spawning gravel and to intergravel rates of flow yield categorically different results. The relative uncertainty of the components of this model indicates that the greatest research needs are to understand how sediment transport affects the intergravel environment and how these changes affect embryo development and survival.

Nous présentons un modèle qui simule les effets de l'écoulement d'un cours d'eau et du transport des sédiments sur la survie des embryons de salmonidés en incubation dans des frayères de gravier dans un chenal naturel. Les composantes du modèle incluent un relevé du débit du cours d'eau pendant 6 ans, une fonction empirique de transport des sédiments de fond, une relation entre le transport et l'infiltration de la charge de fond sableuse dans une couche de gravier, les effets de l'infiltration de sédiments fins sur les propriétés du gravier et des fonctions précédentes qui mettent en relation la survie des embryons et les propriétés du gravier. Les épisodes de débit élevé entraînent des variations temporelles de la survie; les variations à travers le chenal du transport des sédiments de fond causent des variations spatiales. Par conséquent, la survie prévue varie considérablement d'une année à l'autre et d'une montaison de fraye à l'autre au cours de la même année. D'autres fonctions établies lors d'études précédentes qui mettent en relation la survie et la concentration de sédiments fins dans le gravier de la frayère ou l'écoulement entre les graviers donnent des résultats totalement différents. L'incertitude relative des composantes de ce modèle indique que le besoin le plus pressant sur le plan de la recherche est la compréhension de la manière dont le transport des sédiments influe sur l'environnement du gravier et des répercussions de ces changements sur le développement et la survie des embryons.

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Survival of salmonid embryos to emergence from the streambed has been related to substrate and flow conditions in many experiments (Phillips et al. 1975; Tappel and Bjornn 1983; among others) and field studies (Koski 1966; Tagart 1984; Scrivener and Brownlee 1989). The suitability of incubation habitat ultimately depends on how much, what size, and when sediment is transported. Almost regardless of the original condition of gravel, the spawning female can alter the grain size and porosity of gravel to ensure that the ova begin with an adequate flow of oxygenated water (Burner 1951; Cordone and Kelley 1961; among others). For many regions and species, however, incubation coincides with seasonal high flows that carry sediment. Accumulated fine sediment in the gravel can restrict intergravel flow and block emergence of fry. Therefore, the key to embryo survival is not the condition of spawning gravel before or immediately after spawning but during the several weeks or months of incubation. Moreover, stream temperature can control the rate of development and thereby determine the period of incubation (Heggberget 1988). Processes that change gravel conditions vary widely in time and space because they are driven by climatic events and modified

by the complexity of natural channels.

Are effects of sediment transport sufficiently intensive, pervasive, and frequent to significantly affect embryo survival? To answer this question, we need to know how a hierarchy of processes leading from (1) water discharge to (2) sediment transport to (3) changes in gravel conditions to (4) physiologic functions of embryos are linked. We also need to know how the variability of processes affects survival of the population of embryos incubating in a stream. This problem can be approached effectively by modeling if relationships between processes are quantifiable and data are available.

We present a model that links variations in flow and sediment transport to the fraction of salmonids that survive in a natural channel. We do not intend it to serve as a management tool, but as a framework to build more accurate and comprehensive models, reveal gaps in understanding, and explore the temporal and spatial variability of sediment effects on embryo survival in a natural stream. Furthermore, we do not presuppose that quality of incubation habitat limits fish production. Rather, we offer a mechanistic approach to deciphering the physical-biological linkage for one critical life stage of salmonids in streams.

Study Site

We constructed the model with data primarily from one natural stream. Some site-specific relations are included pro tem until more general relations can be found. We applied the model to one reach where data are available; thus the model does not evaluate embryo survival rates for the entire stream.

Jacoby Creek is a small gravel-bed stream that drains into Humboldt Bay near Arcata, California. It supports populations of steelhead trout (*Oncorhynchus mykiss*) and coho salmon (*O. kisutch*) and has been the site of life-history studies of salmonids (Harper 1980), fluvial geomorphology (Lisle 1986), and sediment transport and its effects on spawning gravels (Lisle 1989). Mean bankfull channel width at the primary study reach is 17.2 m, median grain size of the bed surface is 22 mm, and channel gradient is 0.0063. Drainage area is 36.3 km², and peak discharges can exceed 40 m³/s during the rainy season from October through April. Clastic sediment yield (180 tons (t)·km⁻²·yr⁻¹), of which approximately 15% is bedload (Lehre and Carver 1985), is moderate compared with other basins in northwestern California (Janda and Nolan 1979). Large fluxes of sediment during stormflow periods in Jacoby Creek can cause extensive scour and fill and fine-sediment infiltration (Lisle 1989).

Model

The following provides a brief conceptual overview of our model. We assume that adult fish are equally likely to enter Jacoby Creek to spawn each day that discharge is between 0.85 and 4.8 m³/s during the period from January 1 to April 5. In the process of burying the eggs, the females reduce the fraction of fine sediment in the spawning gravel. The embryos then incubate for 40 d, during which periodic high flows transport fine sediment over the bed surface. Some sediment infiltrates into the interstices of the gravel that overlies the embryos, reducing intergravel flow velocities and plugging gravel interstices. These changes increase mortality of the embryos. Our observations in Jacoby Creek suggest that redd topography is planed off by flows large enough to transport significant quantities of sediment. We assume, therefore, that redd topography does not affect infiltration of fine sediment.

Water Discharge

Water discharge drives changes in spawning gravels and is the source of temporal variability of factors that affect embryo survival. We used water stage, recorded continuously at a gauging station at the downstream end of the study reach, to construct a record of discharge from 1979 to 1985. Discharge during the rainy season ranged from 0.4 to 62 m³/s, and associated sediment transport ranged from a trace to hundreds of tons per day. We used data for the period from January 1 to May 15, which includes most of the rainy season. Calculations were initiated after discharge first exceeded 0.85 m³/s each winter, when it was assumed that adults entered the stream to spawn.

Sediment Transport

Sand and granules (0.25 < *D* < 4 mm, where *D* is particle diameter) are the predominant sizes that infiltrate spawning gravels in Jacoby Creek (Lisle 1989). These particle sizes are large enough to be transported in frequent contact with the bed and small enough to enter interstices of spawning gravels.

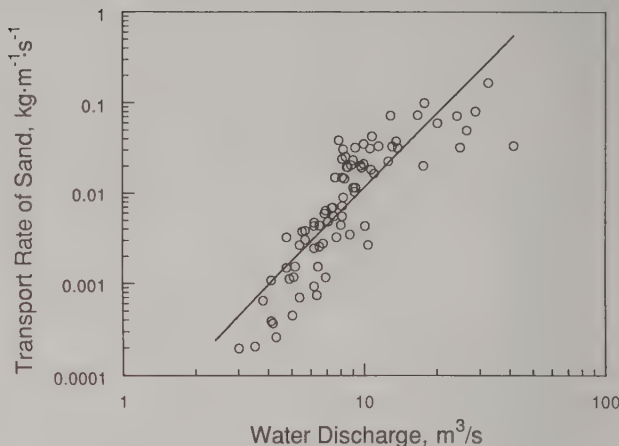


FIG. 1. Relation between transport rate of sand (0.25 < *D* < 2 mm) and water discharge, Jacoby Creek.

Transport rates of this fine bedload were measured directly from a bridge at the gauging station with a Helley-Smith bedload sampler that had a mesh size of 0.20 mm. Fine-bedload transport rate increased steeply with discharge and had wide scatter (Fig. 1) typical of bedload data from natural streams (Gomez 1991). As a point of reference, a transport rate of 0.023 kg·m⁻¹·s⁻¹ equates to 1 t·m⁻¹·d⁻¹.

We used a power function of transport rate versus water discharge to compute fine-bedload fluxes every hour during the modeled period:

$$(1) \quad q_b = (2.19 \times 10^{-5})Q^{2.72} \quad r^2 = 0.71$$

where q_b is mean bedload transport rate per unit channel width (kilograms per metre per second) and Q is water discharge (cubic metres per second). We computed mean unit flux of fine bedload (kilograms per metre) by summing equation (1) for each 40-d incubation period.

Infiltration of Fine Sediment

The volume of fine sediment that accumulates in spawning gravels in Jacoby Creek is related to the flux of fine bedload (Lisle 1989). Cans filled with gravel devoid of fine sediment were buried in spawning areas to measure rates and grain sizes of fine-sediment infiltration. After periods in which sediment transport was measured continuously, we emptied the cans and sieved their contents. For the model, infiltrated sediment was considered to range between 0.12 and 4.0 mm in diameter, which represents 93% of the material finer than 4 mm collected in the cans. We considered this as the maximum size range that we could use to determine infiltration rates from transport rates of sediment between 0.25 and 4 mm.

Sediment infiltration rates were related to bedload transport rates by a power function (Lisle 1989) modified to include a wider range of grain size than used originally:

$$(2) \quad I = 2.03(q_b)_T^{0.412}$$

where I is mean fine-sediment infiltration (kilograms per square metre of spawning gravel) and $(q_b)_T$ is fine-bedload flux per unit width (kilograms per metre). Data from two other streams were also used to derive the original function (Lisle 1989). All three streams have unimodal bed material and each shows no systematic deviation from the common relationship between

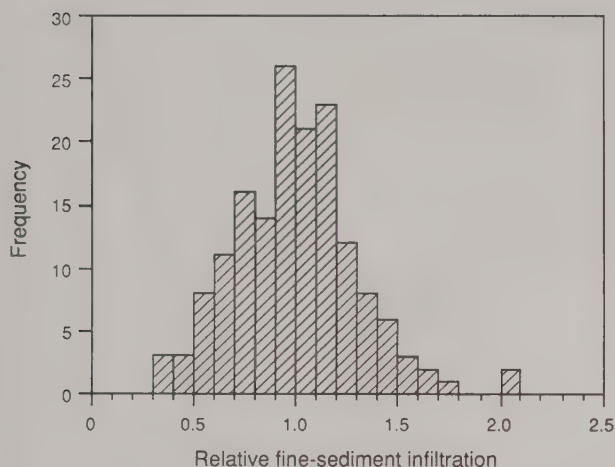


FIG. 2. Frequency distribution of relative fine-sediment infiltration rates (fine-sediment content in each gravel can divided by the mean of all cans for corresponding measurement periods) during stormflows, Jacoby Creek.

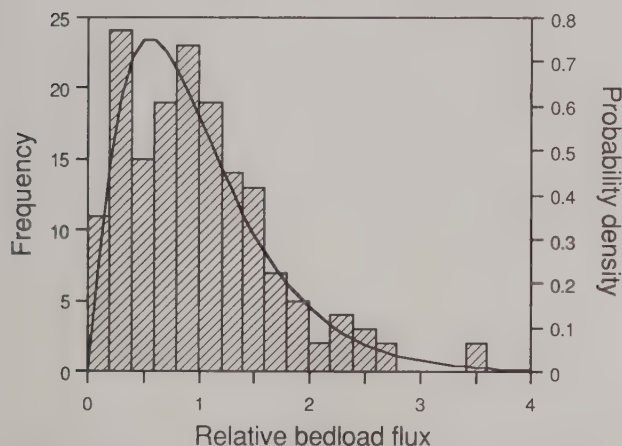


FIG. 3. Frequency and probability density distribution of relative bedload fluxes (local flux divided by mean flux), Jacoby Creek.

sediment infiltration and transport. Equation (2) was used to compute fine-sediment infiltration for each incubation period.

Fine-sediment infiltration into the gravel cans varied widely within each stormflow period (Fig. 2). To explicitly incorporate this variation in the model, we assumed that it was caused solely by spatial variation in bedload transport. Bedload transport characteristically varies widely across channels (Carey 1985; Emmett, cited in Gomez 1991; Gomez et al. 1991). Equation (2) indicates that infiltration rate for a given sediment transport rate decreases as total sediment flux increases. The physical explanation is that surficial interstices are free of fine sediment during initial stages of infiltration and become plugged as infiltration progresses, thereby inhibiting further infiltration. A greater proportion of transported sediment can infiltrate areas of the bed with low accumulated sediment fluxes more readily than areas where high fluxes have previously plugged many surficial interstices. This tends to dampen differences between areas of the streambed in total sediment infiltrated.

We inverted equation (2) to compute local bedload fluxes from values of infiltration measured in 153 cans that were

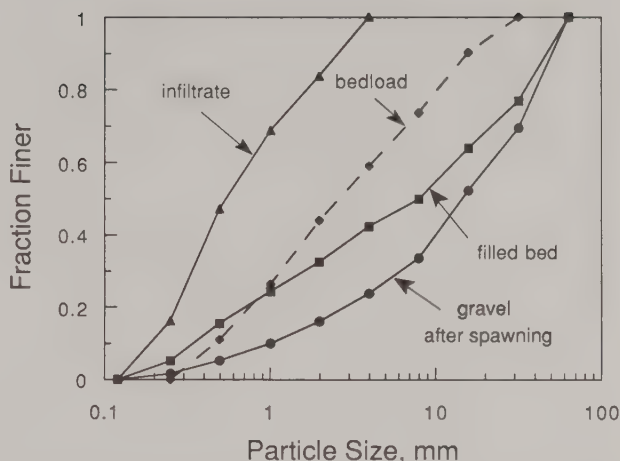


FIG. 4. Cumulative size distributions by weight of fine-sediment infiltration, bedload, spawning gravel whose interstices are completely filled with fine sediment, and spawning gravel immediately after spawning, Jacoby Creek.

recovered from five transects after six measured stormflow periods. Values of local flux were normalized by dividing by the mean value of flux for each measurement period, and the probability density distribution of normalized fluxes was fit to a gamma distribution (Fig. 3):

$$(3) \quad f[(q_B)_T] = 5.351[(q_B)_T]^{1.232} e^{-2.232(q_B)_T}.$$

The discharge record and equations (1) through (3) could be used to generate local sediment fluxes and infiltration rates for each incubation period.

Changes in Spawning Gravel Conditions

We modeled effects of fine-sediment infiltration on gravel properties that decrease embryo survival by two alternative approaches: (1) an increase in the fraction of fine sediment in the gravel, which affects both the rate of flow of oxygenated water to the embryos and the ability of emerging fry to penetrate the gravel interstices, and (2) a reduction in permeability of the gravel, which decreases intergravel flow velocities.

In both approaches, we start with the average grain size distribution of Jacoby Creek spawning gravels, which contain a small residual fraction of fine sediment, and recalculate gravel properties at the end of each incubation period. We do not, therefore, distinguish between adverse conditions that may prevail throughout most of an incubation period and those that may accrue only near the end of the period. The particle-size distribution of gravel overlying freshly spawned eggs is the average distribution obtained from freeze-core samples at five spawning areas (Lisle 1989) minus a proportion of the fraction finer than 4 mm such that the fraction finer than 1 mm equals 10% (Fig. 4). Initial porosity of the gravel is 0.35. The thickness of the gravel layer is 0.2 m, which approximates the average burial depth of eggs of steelhead trout and coho salmon (Shapalov and Taft 1954; Van den Berghe and Gross 1984).

The framework of the gravel bed after spawning is assumed to remain intact as fine sediment fills the interstices. That is, neither scour and fill nor expansion of the gravel framework occurs. Particle-size distribution of fine sediment between 0.12 and 4 mm was obtained from the average distribution of material collected in the cans (Fig. 4). The disregarded fraction finer

than 0.12 mm constituted only 7% of the material finer than 4 mm. Particle-size distribution of bedload material does not equal that of infiltrated fines, partly because the distributions are not truncated at the same minimum values. Also, fine bedload particles can more easily enter gravel interstices than coarse particles and are thus disproportionately represented in sediment infiltrating the gravel (Einstein 1970; Carling 1984; Lisle 1989).

The introduction of fines changes the value of grain-size parameters used in the model. In approach 1, bed-material fractions finer than 1 mm and finer than 8 mm were calculated for increasing values of accumulation. These grain-size ranges correspond approximately to those used by Tappel and Bjornn (1983) to relate survival to emergence of four species of salmonids.

In approach 2, effective diameter, which is used in calculations of permeability, is reduced by fine-sediment infiltration. Effective diameter is defined by

$$(4) \quad D_e = [\text{sum}(p_i / \psi_i D_i)]^{-1}$$

where p_i is the volume fraction and ψ_i is the sphericity of particles of size D_i (Johnson 1980). Sphericity is given a value of 0.7.

Porosity is also reduced as interstices are filled with sediment. Given initial conditions, porosity (E) is calculated as a function of fine-sediment infiltration (I , kilograms per square metre):

$$(5) \quad E = E_0 - E_0(1 - E_f)(I/I_{\max})$$

where $E_0 = 0.35$ is initial porosity, $E_f = 0.40$ is porosity of fine sediment, and $I_{\max} = 0.042 \text{ kg/m}^2$ is fine-sediment accumulation to the point where interstices are completely filled. Mass of sediment was converted to volume by dividing by a particle density of 2.5 g/cm^3 that was measured from Jacoby Creek sediment. Using these values, equation (5) simplifies to

$$(6) \quad E = 0.35 - 5.0I.$$

$E = 0.14$ when interstices are completely filled. This value corresponds closely to those for undisturbed gravel beds measured by Carling and Reader (1982).

Permeability (K) is calculated from the Karman-Cozeny equation (Scheidegger 1960):

$$(7) \quad K = gf(E)D_e^2/36\kappa\nu$$

where K is in centimetres per second, g is gravitational acceleration, $f(E) = E^3/(1 - E)^2$, and ν is kinematic viscosity. κ is an empirically derived permeability constant and is given a value of 6.4, which Johnson (1980) found suitable for spawning gravel.

Apparent intergravel flow velocity (u_a) is calculated from Darcy's Law:

$$(8) \quad u_a = K(dh/dl)$$

where u_a is in centimetres per second. Head loss (dh/dl) over riffle crests where fish commonly spawn is given a value of 0.005. Channel gradient of the study reach equals 0.0062. We use values of u_a to compute expected survival.

Embryo Survival

We use the two approaches for evaluating changes in gravel properties (described above) and corresponding relations to embryo survival (described below) to explicitly evaluate uncertainty in relating survival to gravel properties.

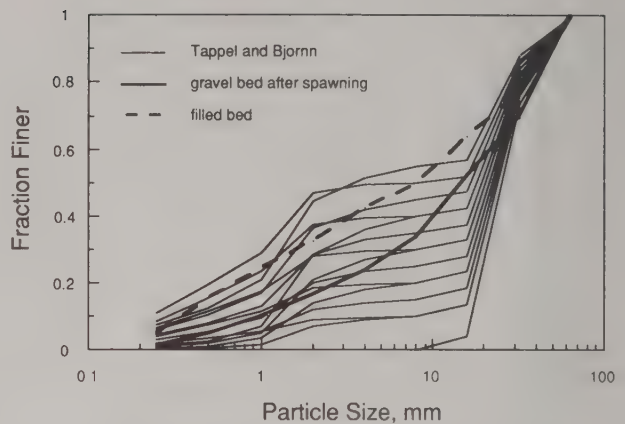


FIG. 5. Cumulative frequency distributions of particle sizes used in our model and in experiments by Tappel and Bjornn (1983).

In the first approach, we use Tappel and Bjornn's (1983) empirical relation between survival of steelhead embryos and the percentages of spawning gravel finer than 0.85 mm and finer than 9.5 mm:

$$(9A) \quad S = 94.7 - 0.1P_{9.5}P_{0.85} + (P_{9.5})^2$$

where S is expected percent survival to emergence and $P_{9.5}$ and $P_{0.85}$ are the percent volume of sediment finer than 9.5 and 0.85 mm, respectively, in the overlying gravel. We substitute P_8 for $P_{9.5}$ and P_1 for $P_{0.85}$. Although Tappel and Bjornn's (1983) empirical relation fits specific laboratory conditions, we justify its use in our model by comparing their gravel properties with those used in our model. First, we both use a burial depth of 0.2 m. Second, their particle-size distributions of gravel-sand mixtures overlap our starting distribution and the distribution corresponding to maximum infiltration (Fig. 5).

Expected survival computed for a range of values of fine-sediment infiltration fits a linear relation well:

$$(9B) \quad S = 67.9 - 0.648I \quad r^2 = 0.999$$

which, when combined with equation (2), yields

$$(9C) \quad S = 67.9 - 1.10(q_B)_T^{0.412}.$$

In our second approach, survival is a function of intergravel flow velocity according to an empirical relation derived from data for sockeye salmon (J. Pyper, cited in Cooper 1965):

$$(10A) \quad S = 167.0 + 46.3\log(u_a) \quad r^2 = 0.96.$$

The wall thickness and diameter of steelhead eggs are usually smaller than those of sockeye salmon; thus, demands for oxygen concentration for steelhead should be greater (Wickett 1975; Beacham and Murray 1989). Consequently, equation (10A) should overestimate survival of steelhead embryos.

Survival then approximates a linear function of infiltration which is substituted for u_a using equations (4), (6), (7), and (8):

$$(10B) \quad S = 56.8 - 0.918I \quad r^2 = 0.999.$$

Then by using equation (2) to substitute $(q_B)_T$ for I :

$$(10C) \quad S = 56.8 - 1.864(q_B)_T^{0.412}.$$

For a given sediment flux, the survival function generated from the Tappel and Bjornn (1983) relation (9C) yields higher rates of survival than that generated from the intergravel-flow function (10C) (Fig. 6). This discrepancy is especially large for

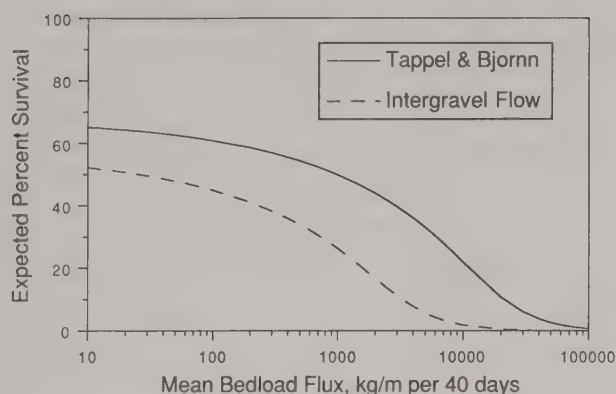


FIG. 6. Functions of embryo survival versus bedload flux (equations (9C) and (10C)) using the intergravel flow function and Tappel and Bjornn's (1983) relation.

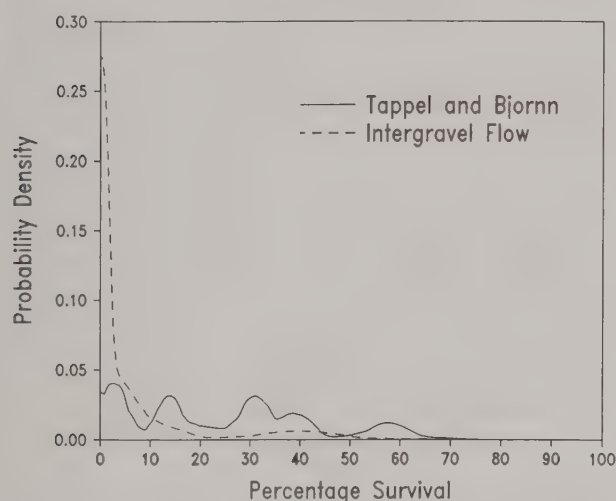


FIG. 7. Probability density distribution of embryo survival for all 40-d incubation periods over a 6-yr period using the intergravel-flow function and Tappel and Bjornn's (1983) relation.

fluxes between 5000 and 20 000 kg/m, a range that was commonly achieved during the 40-d periods.

Because each 40-d summation of mean sediment flux from equation (1) is associated with a distribution of values of local flux, expected survival must be integrated over equation (3) using equations (9C) and (10C). This results in a distribution of survival rates for each value of mean flux.

Results

Probability density distributions of expected embryo survival were compiled for the entire period of record using the Tappel-Bjornn and intergravel-flow functions (Fig. 7). Expected survival for each 40-d incubation period is the mean value resulting from mean sediment flux and spatial variations in sediment flux and infiltration. Variations in expected survival arise from temporal variation in sediment transport due to climatic events. The salient feature of both distributions is the high variability of survival rates.

The two survival functions yield categorically different survival rates. Results from the Tappel-Bjornn function indicate

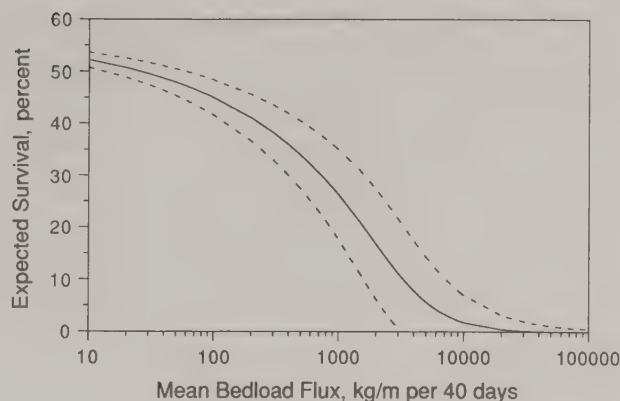


FIG. 8. Integrated relation computed from the intergravel-flow function between expected embryo survival and mean bedload flux with ± 1 SD in survival about the mean due to spatial variations in fine-sediment transport and infiltration.

that survival rates for each day eggs are deposited would most often exceed 10%. In contrast, most of the distribution of survival rates computed from the intergravel-flow function falls below 5%. Depending on critical embryo survival rates necessary to maintain fish populations, one could conclude that the quality of incubation habitat does or does not limit populations, depending on the choice of survival functions.

Spatial variations in sediment transport and infiltration create high variations in expected survival, particularly for high values of sediment flux. The standard deviation of expected survival, expressed as either an absolute value or a ratio to mean expected survival (coefficient of variation), increases with increasing mean sediment flux computed from the intergravel-flow function (Fig. 8). The coefficient of variation is greatest for mean values of flux exceeding approximately 5000 kg/m and corresponding to mean survival rates of less than 10%. Given that such low survival rates are realized most of the time according to this function (Fig. 7), high spatial variations typify expected survival in Jacoby Creek.

Temporal variations in expected survival are also high. Embryo survival rates can be expected to vary widely from year to year because of the episodic nature and strong influence of stormflows that punctuate the spawning and incubation season (Fig. 9). In some years, e.g. 1983, high-flow events are frequent, while in others, e.g. 1985, only one event may occur. Even in a relatively dry year, e.g. 1980, survival may be low if all eggs are laid over a short period and a single high-flow event happens to occur during incubation.

Discussion

Evaluation of Uncertainty and Variability

One of the most useful immediate results of modeling a system of natural processes is an evaluation of the relative uncertainty and variability of individual processes. As more knowledge of a process is gained and uncertainty diminishes, our awareness of its variability often increases. Among the hierarchy of processes between sediment transport and salmonid embryo survival, variability is high and uncertainty relatively low in the driving processes (streamflow and sediment transport), and uncertainty is high in the response processes (changes in gravel conditions and embryo mortality).

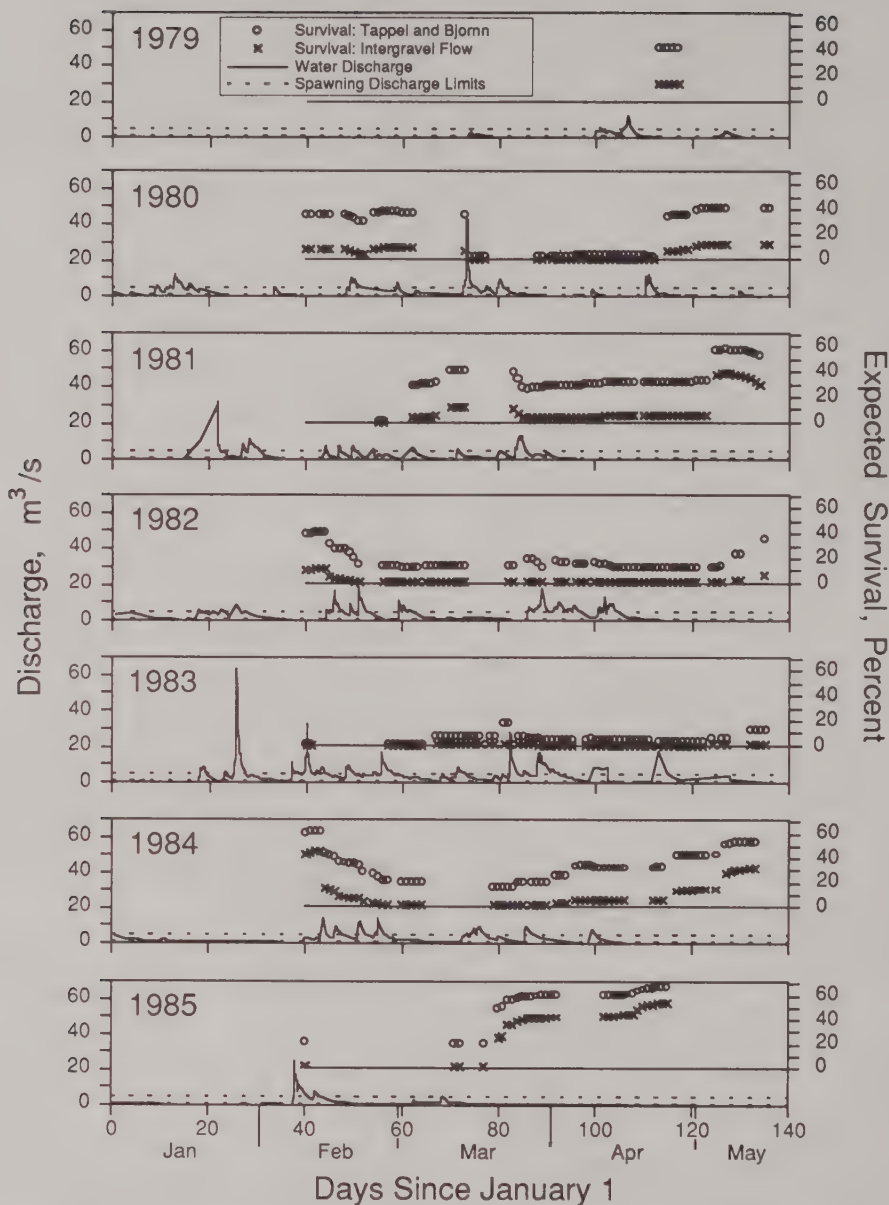


FIG. 9. Hydrographs and expected survival of embryos for the period of January 1 to May 15 for the years 1979–85. Plots of survival correspond to the day of emergence of alevins after a 40-d incubation period following spawning which occurs within discharge limits of 0.85 and 4.8 m³/s.

Streamflow is highly variable but measured routinely with little uncertainty. Bedload transport varies approximately with streamflow, and varies considerably laterally and longitudinally during a stormflow event (see Gomez 1991). Bedload transport rates can be measured directly with careful attention to sampling strategies and limitations of samplers or predicted with an expected accuracy of within an order of magnitude (Gomez and Church 1989). Considerable investment in measurement and modeling is required to quantify spatial variation in bedload transport. Compared with its effects on gravel properties and embryo survival, however, variability in bedload transport outweighs its uncertainty.

Uncertainty in effects of sediment transport on spawning gravel is high because of a lack of understanding of the variation of fine-sediment infiltration and scour and fill with hydraulic and sedimentological properties of streams. Scour and fill of streambeds can alter streambed composition as much as sediment infiltration (Lisle 1989) and can also result in fatal entrainment of embryos (Gangmark and Broad 1956). Although mean depths of scour and fill in gravel-bed channels have been modeled (Ziemer et al. 1991), such models are crude and do not adequately incorporate spatial variations. Relations between sediment transport and fine-sediment infiltration are either empirical, and thus applicable only to channels similar to those

where relations were developed, or fit stringent sedimentological criteria. Where sand is abundant, rates of infiltration are influenced by plugging of gravel interstices near the bed surface early in the infiltration process, which inhibits further infiltration into deeper layers (Beschta and Jackson 1979; Carling 1984; Lisle 1989; Diplas and Parker 1992). Alonso et al. (1988) developed a model of sediment infiltration and its effects on gravel properties in a channel containing abundant silt and clay, but little sand. Under these conditions, silt and clay are able to penetrate all but the smallest interstices and thus fill beds from the bottom up (Einstein 1968).

Uncertainty in the depth of fine-sediment infiltration complicates modeling of changes in gravel conditions and their effect on embryo survival. Empirical relations between survival of eggs to emergence and the volume of fine sediment deposited in redds vary considerably depending on grain size distributions of fines and redd material, distribution of fines with depth in the bed, and other conditions imposed in the laboratory or in the field (Everest et al. 1987; Chapman 1988). As a result, there are no clear criteria to choose between relations for a model such as ours.

Chapman (1988), for example, objected to the application of relations developed under laboratory conditions to field situations on the grounds that gravel conditions in natural egg pockets are poorly understood and not replicated by the uniform distributions of fines with depth imposed in laboratory experiments. The implication, however, that the greater permeability that has been observed in an egg pocket (Platts and Penton 1980) translates to a greater rate of intergravel flow than indicated by the material surrounding the pocket may also be erroneous. Subsurface streamlines do converge into a permeable volume, but only when inflow and outflow conduits exist. Thus, intergravel flow through a permeable egg pocket may be governed more by conditions in the surrounding material than those in the pocket itself. As Chapman (1988) admonished, the true nature of bed material structure associated with redds and the effects of sedimentation on embryo survival in natural channels are not well understood and need to be improved before accurate, predictive modeling can proceed. The categorically different results from our model resulting from two applicable survival functions highlight this need.

The issue is further complicated by multiple causes of mortality, as well as adaptations of ova and alevins that increase survival under low rates of intergravel flow and low levels of dissolved oxygen (reviewed in Everest et al. 1987). Even if intergravel flow is adequate for embryo development, sand that plugs near-surface interstices can prevent alevins from emerging from the gravel (Koski 1966; Phillips et al. 1975). Degradation of incubation conditions can also lead to smaller size of alevins (Silver et al. 1963; Shumway et al. 1964; Bams 1969), which may decrease chances for survival of fry. The immediate cause of mortality from reduced intergravel flow is the reduction of oxygen available to the embryos. Oxygen concentration of intergravel water does not necessarily depend on intergravel flow velocity, but they usually correlate (Chapman 1988). Fine sediment in Jacoby Creek gravel contains abundant fine particulate organic matter. Subsurface samples of gravel whose interstices were filled with fine sand and silt smelled of decaying organics, suggesting that low oxygen concentrations are associated with high concentrations of fine sediment.

Influence of Diversity and Size of Spawning Runs

The strong influence of individual stormflow events suggests that young-of-the-year fish populations would vary less widely

if spawning were spread out in time over the rainy season. This is most likely to result from a diversity of species, races, and genotypes, as well as high escapement. Steelhead, for example, spawn late in the season during the onset of drier weather in many streams, and their embryos would have an advantage if high flows became less frequent. Reductions in diversity of genotypes that lead to concentrated spawning over a short period would seem to threaten fish populations because even in relatively dry years the annual recruitment could be threatened by a single stormflow. The number of embryos surviving each year to provide annual fry recruitment is more likely to affect the dynamics of fish populations than the composite survival rate over a period of years.

Similarly, wide spatial variations in sediment effects suggest that a diversity of redd sites can promote stability in fry recruitment. If a large number of redds are constructed, the few located in zones of low sediment flux and infiltration may produce enough fry to compensate for high mortality elsewhere. Steelhead do not appear to select low-risk spawning sites in Jacoby Creek but instead choose loose gravel of the appropriate size often in the thalweg. Our measurements have shown that sediment transport and scour and fill are high in such areas (Lisle 1989).

Fewer redds, resulting from reduced size and diversity of spawning runs, would lead to increased variability in embryo survival and a greater risk of dangerously low populations even in the most favorable of seasons. As populations of emerging fry become less, embryo survival rates become critical for fish populations if the available rearing habitat for juveniles is not adequately filled. At the same time, spawning success becomes less predictable on the basis of the quality of incubation habitat for embryos.

Conclusions

Despite a considerable degree of uncertainty and inherent variability, the effects of flow and sediment transport on salmonid embryo survival can be modeled. Spatial and temporal variability of water discharge, sediment transport, and fine-sediment infiltration can be explicitly incorporated. Relations between driving and response variables can be quantified to various degrees of generality, precision, and accuracy. The resulting model is sufficiently comprehensive to explore variability, highlight research needs, and perhaps evaluate relative effects of changes in sediment transport regime.

Regardless of the uncertainties in many of the processes we modeled, it is clear that the fraction of embryos surviving to emerge from spawning gravels each year can be expected to be extremely variable in streams like Jacoby Creek, where fish spawn during the high-flow season and the channel bed is at least moderately mobile. In these cases, and especially where adult escapement is low (Everest et al. 1987), the nature of the linkage between the sediment transport regime and embryo mortality may be to limit fish production in some years but not others.

Some research needs are clear. The further we move down the hierarchy of processes from water discharge to embryo mortality the more we find is unknown. Relations between sediment transport and gravel conditions need to become more general, include scour and fill, and adequately treat variations in depth and grain size of fine-sediment infiltration. More crucially, the subsurface structure of natural redds and the response of embryos to conditions in spawning gravels need to be quantified better. Progress in the problems outlined above will move

us closer to predicting embryo survival under changing watershed conditions and improve our understanding of factors influencing population dynamics of stream fishes.

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References

- ALONSO, C. V., F. D. THEURER, AND D. W. ZACHMANN. 1988. Tucannon River offsite study: sediment intrusion and dissolved-oxygen transport model. USDA, Agricul. Res. Serv., Hydro-Ecosystems Res. Group, Fort Collins, CO.
- BAMS, R. A. 1969. Adaptions of sockeye salmon associated with incubation in stream gravels, p. 71–87. *In* T. G. Northcote [ed.] Symposium on salmon and trout in streams, H. R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B.C.
- BEACHAM, T. D., AND C. B. MURRAY. 1989. Variation in developmental biology of sockeye salmon (*Oncorhynchus nerka*) and chinook salmon (*O. tshawytscha*) in British Columbia. *Can. J. Zool.* 67: 2081–2089.
- BESCHTA, R. L., AND W. L. JACKSON. 1979. The intrusion of fine sediments into a stable gravel bed. *J. Fish. Res. Board Can.* 36: 204–210.
- BURNER, C. J. 1951. Characteristics of spawning nests of Columbia River salmon. *U.S. Fish Wildl. Serv. Fish. Bull.* 52(61): 97–110.
- CAREY, W. P. 1985. Variability in measured bedload-transport rates. *Water Resour. Bull.* 21(1): 39–48.
- CARLING, P. A. 1984. Deposition of fine and coarse sand in an open-work gravel bed. *Can. J. Fish. Aquat. Sci.* 41: 263–270.
- CARLING, P. A., AND N. A. READER. 1982. Structure, composition and bulk properties of upland stream gravels. *Earth Surf. Proc. Landforms* 7: 349–365.
- CHAPMAN, D. W. 1988. Critical review of variables used to define effects of fines in redds of large salmonids. *Trans. Am. Fish. Soc.* 117(1): 1–21.
- COOPER, A. C. 1965. The effect of transported stream sediments on the survival of sockeye and pink salmon eggs and alevin. *Int. Pac. Salmon Fish. Comm. Bull.* 17: 71 p.
- CORDONE, A. J., AND D. W. KELLEY. 1961. The influences of inorganic sediment on the aquatic life of streams. *Calif. Fish Game* 47(2): 189–228.
- DIPLAS, P., AND G. PARKER. 1992. Deposition and removal of fines in gravel-bed streams. *In* P. Billi, R. D. Hey, C. R. Thorne, and P. Tacconi [ed.] Dynamics of gravel-bed rivers. *J. Wiley & Sons, Ltd., Chichester, U.K.* (In press)
- EINSTEIN, H. A. 1968. Deposition of suspended particles in a gravel bed. *J. Hydraul. Div. ASCE* 94(HY5): 1197–1205.
1970. Closure, Deposition of suspended particles in a gravel bed. *J. Hydraul. Div. ASCE* 96(HY2): 581–582.
- EVEREST, F. H., R. L. BESCHTA, J. D. SCRIVENER, K. V. KOSKI, J. R. SEDELL, AND C. J. CEDERHOLM. 1987. Fine sediment and salmonid production — a paradox, p. 98–142. *In* E. Salo and T. Cundy [ed.] Streamside management and forestry and fishery interactions. University of Washington, College of Forestry Resources, Cont. 57, Seattle, WA.
- GANGMARK, H. A., AND R. D. BROAD. 1956. Further observations on stream survival of king salmon spawn. *Calif. Fish Game* 42: 37–49.
- GOMEZ, B. 1991. Bedload transport. *Earth-Sci. Rev.* 31: 89–132.
- GOMEZ, B., AND M. CHURCH. 1989. An assessment of bed load sediment transport formulae for gravel bed rivers. *Water Resour. Res.* 25(6): 1161–1186.
- GOMEZ, B., W. W. EMMETT, AND D. W. HUBBELL. 1991. Comments on sampling bedload in small rivers. *Proc. 5th Fed. Inter-Agency Sediment. Conf.* 1: 2.65–2.72.
- HARPER, W. G. 1980. Age, growth, and migration of coho salmon and steelhead trout in Jacoby Creek, California. M.S. thesis, Humboldt State University, Arcata, CA. 103 p.
- HEGGBERGET, T. G. 1988. Timing of spawning in Norwegian Atlantic Salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 45: 845–849.
- JANDA, R. A., AND K. M. NOLAN. 1979. Stream sediment discharge in northwestern California, p. IV-1 to IV-27. *In* Natural and management-related erosion in Franciscan Terrane of northern California. *Geol. Soc. Am. Cordilleran Sect. Guidebook, Field Trip 12.* San Jose State University, San Jose, CA.
- JOHNSON, R. A. 1980. Oxygen transport in salmon spawning gravels. *Can. J. Fish. Aquat. Sci.* 37: 155–162.
- KOSKI, K. V. 1966. The survival of coho salmon (*Oncorhynchus kisutch*) from egg deposition to emergence in three Oregon coastal streams. M.S. thesis, Oregon State University, Corvallis, OR. 84 p.
- LEHRE, A. K., AND G. CARVER. 1985. Thrust faulting and earthflows: speculations on the sediment budget of a tectonically active drainage basin, p. 169–182. *In* M. E. Savina [ed.] Redwood Country, American Geomorphological Field Group 1985 Field Trip Guidebook, Humboldt State University, Arcata, CA.
- LISLE, T. E. 1986. Stabilization of a gravel channel by larve streamside obstructions and bedrock bends, Jacoby Creek, northwestern California. *Geol. Soc. Am. Bull.* 97: 999–1011.
1989. Sediment transport and resulting deposition in spawning gravels, north coastal California. *Water Resour. Res.* 25(6): 1303–1319.
- PHILLIPS, R. W., R. L. LANTZ, E. W. CLAIRE, AND J. R. MORING. 1975. Some effects of gravel mixtures on emergence of coho salmon and steelhead trout fry. *Trans. Am. Fish. Soc.* 104(3): 461–466.
- PLATTS, W. S., AND V. E. PENTON. 1980. A new freezing technique for sampling salmonid redds. *USDA For. Serv. Res. Pap. INT-248.* 22 p.
- SCHEIDEGGER, A. E. 1960. The physics of flow through porous media. Macmillan Co., New York, NY. 313 p.
- SCRIVENER, J. C., AND M. J. BROWNLEE. 1989. Effects of forest harvesting on spawning gravel and incubation survival of chum (*Oncorhynchus keta*) and coho salmon (*O. kisutch*) in Carnation Creek, British Columbia. *Can. J. Fish. Aquat. Sci.* 46: 681–696.
- SHAPALOV, L., AND A. C. TAFT. 1954. The life histories of the steelhead rainbow trout (*Salmo gairdneri gairdneri*) and silver salmon (*Oncorhynchus kisutch*) with special reference to Waddell Creek, California, and recommendations regarding their management. *Calif. Dep. Fish Game Bull.* 98: 375 p.
- SHUMWAY, D. L., D. E. WARREN, AND P. DOUDOROFF. 1964. Influence of oxygen concentration and water movement on the growth of steelhead trout and coho salmon embryos. *Trans. Am. Fish. Soc.* 93(4): 342–456.
- SILVER, S. T., C. E. WARREN, AND P. DOUDOROFF. 1963. Dissolved oxygen requirements of developing steelhead trout and chinook salmon embryos at different water velocities. *Trans. Am. Fish. Soc.* 92(4): 327–343.
- TAGART, J. V. 1984. Coho salmon survival from egg deposition to fry emergence, p. 173–181. *In* J. M. Walton and D. B. Houston [ed.] Proc. Olympic Wild Fish Conference. Peninsula College and Olympic National Park, Port Angeles, WA.
- TAPPEL, P. D., AND T. C. BJORN. 1983. A new method of relating size of spawning gravel to salmonid embryo survival. *N. Am. J. Fish. Manage.* 3: 123–135.
- VAN DEN BERGHE, E. P., AND M. R. GROSS. 1984. Female size and nest depth in coho salmon (*Oncorhynchus kisutch*). *Can. J. Fish. Aquat. Sci.* 41: 204–206.
- WICKETT, W. P. 1975. Mass transfer theory and the culture of fish eggs. *In* W. Adams [ed.] *Trans. Electrochem. Soc. Princeton, NJ* 419–433.
- ZIEMER, R. R., J. LEWIS, R. M. RICE, AND T. E. LISLE. 1991. Modeling the cumulative watershed effects of forest management strategies. *J. Environ. Qual.* 20(1): 36–42.

Salmonid Phylogeny Inferred from Ribosomal DNA Restriction Maps

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Phillips, R. B., K. A. Pleyte, and M. R. Brown. 1992. Salmonid phylogeny inferred from ribosomal DNA restriction maps. *Can. J. Fish. Aquat. Sci.* 49: 2345–2353.

Genomic DNA was isolated from 17 salmonid species including six species of *Salvelinus* (*S. namaycush*, *S. fontinalis*, *S. leucomaenis*, *S. confluentus*, *S. malma*, and *S. alpinus*), two species of *Salmo* (*S. trutta* and *S. salar*), eight species of *Oncorhynchus* (*O. mykiss*, *O. clarki*, *O. masou*, *O. tshawytscha*, *O. kisutch*, *O. keta*, *O. nerka*, and *O. gorbuscha*), and one species of *Hucho* (*H. perryi*). Restriction maps of the ribosomal DNA were prepared by using probes to the 18S and 28S coding regions of the rDNA. Phylogenies were constructed from this data using both cladistic and distance methods. Results supported the recent placement of the Pacific trouts in the genus *Oncorhynchus*. The phylogeny obtained for the genus *Salvelinus* suggested that *S. confluentus* from North America may be more closely related to *S. leucomaenis* from Japan than to species in the *S. malma* – *S. alpinus* complex. Fixed differences in restriction sites were found among the major subgroups within the *S. malma* – *S. alpinus* complex.

Nous avons isolé l'ADN génomique de 17 espèces de salmonidés incluant six espèces de *Salvelinus* (*S. namaycush*, *S. fontinalis*, *S. leucomaenis*, *S. confluentus*, *S. malma*, et *S. alpinus*), deux espèces de *Salmo* (*S. trutta* et *S. salar*), huit espèces de *Oncorhynchus* (*O. mykiss*, *O. clarki*, *O. masou*, *O. tshawytscha*, *O. kisutch*, *O. keta*, *O. nerka*, et *O. gorbuscha*), et une espèce de *Hucho* (*H. perryi*). Nous avons préparé des cartes de restriction de l'ADN ribosomique en utilisant des sondes moléculaires correspondant aux régions de l'ADNr codant pour l'ARNr 18S et 28S. À partir de ces données, nous avons construit des relations phylogénétiques en utilisant des méthodes tant cladistiques que de distance. Les résultats corroborent l'inclusion récente des truites du Pacifique dans le genre *Oncorhynchus*. L'étude phylogénétique du genre *Salvelinus* semble indiquer que l'espèce *S. confluentus* d'Amérique du Nord peut être plus étroitement apparentée à l'espèce *S. leucomaenis* du Japon qu'aux espèces du complexe *S. malma* – *S. alpinus*. On a retrouvé des différences fixes entre les sites de restriction des principaux sous-groupes du complexe *S. malma* – *S. alpinus*.

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The subfamily Salmoninae includes six genera of trouts, salmon, and charrs: *Brachymystax*, *Hucho*, *Salmothymus*, *Salvelinus*, *Salmo*, and *Oncorhynchus*. These fishes have been the subjects of numerous taxonomic studies using morphological (Norden 1961; Behnke 1965, 1980, 1989; Cavender 1978, 1980; Cavender and Kimura 1989; Smith and Stearley 1989; Stearley 1990), karyological (reviewed in Hartley 1987; Phillips et al. 1989a, 1989b), ontogenetic (Balon 1980; Pavlov 1980; Kendall and Behnke 1984), allozyme (Tsuyuki and Roberts 1966; Utter et al. 1973; Johnson 1984), and mitochondrial DNA markers (Thomas et al. 1986; Gyllenstein and Wilson 1987; Ginatulina et al. 1988; Grewe et al. 1990). However, there are a number of unresolved problems. Major areas of uncertainty involve the relationships of the three more primitive genera to the others, and the relationships among the species in the genus *Salvelinus* and among the species in the genus *Oncorhynchus* (reviewed in Phillips and Pleyte 1991). Although the three primitive genera *Brachymystax*, *Hucho*, and *Salmothymus* are found only in Europe and

Asia, a Miocene fossil species, *Paleolox larsoni*, which shares many features with *Hucho* and some with *Salvelinus*, has been found in Idaho (Smith et al. 1982).

In the case of the species in the genus *Salvelinus*, the phylogenetic trees based on allozyme and mtDNA data are different from the ones based on osteological data, and unresolved branches remain in all of the trees. For species in the genus *Oncorhynchus*, the tree based on mtDNA conflicts with the trees based on allozymes and osteological data. From the theoretical point of view, one would expect DNA sequence comparisons to be more useful than protein allozymes for phylogenetic analysis, especially in the tetraploid salmonid species. However, it has been shown that maternally inherited mtDNA can "invade" the genome of related species (Ferris et al. 1983) so that a phylogeny based on mtDNA alone may be incorrect. It is likely that hybridization between the various salmonid species has been common in the past, so that examination of nuclear DNA sequences is essential for a more complete systematic analysis.

The nuclear ribosomal RNA genes (rDNA) are especially suitable for use in phylogenetic analysis, as there are three types of regions that evolve at different rates (see Fig. 1). The coding regions for the 5.8S rRNA, the 18S rRNA, and most of the 28S rRNA evolve slowly and can be used in comparisons of distantly related species. The internal and external transcribed spacer regions (ITS-1, ITS-2, 3'ETS, and 5'ETS) as well as

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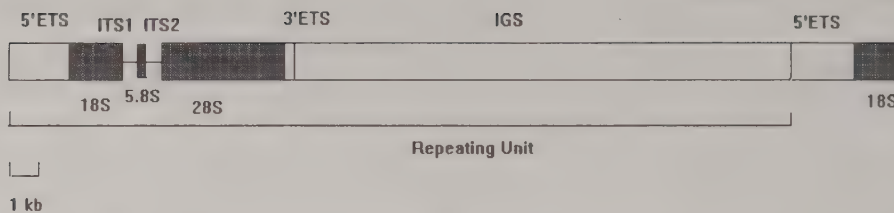


FIG. 1. Diagram showing the structure of the ribosomal RNA genes in eukaryotes. The 5.8S, 18S, and 28S are the regions coding for the 5.8S, 18S, and 28S rDNAs. The ITS-1 and ITS-2 are the internal transcribed spacer regions, the 5'ETS and the 3'ETS are the external transcribed spacer regions, and the IGS is the intergenic spacer region between the repeating units.

the expansion segments of the 28S rRNA evolve more rapidly and can be used in comparison of more closely related species. The intergenic spacer region (IGS) evolves very rapidly and can be used in comparisons of subspecies or populations within a species. Restriction maps of the entire rDNA repeating unit have been used successfully for phylogenetic analysis in several taxa (Coen et al. 1982; Wilson et al. 1984). For example, data obtained from rDNA restriction maps in 32 species of *Rana* that last shared a common ancestor 50 million yr ago have provided an independent test of the previous phylogenetic hypothesis based on allozymes and morphology (Hillis and Davis 1986). In this paper, we present a phylogenetic analysis of restriction site data on the nuclear ribosomal DNA in salmonid fishes of four genera: *Hucho*, *Salvelinus*, *Salmo*, and *Oncorhynchus*.

Materials and Methods

Tissue samples were obtained from individuals of 17 different species, with at least two geographically distinct populations sampled for all of the species except those native to Japan and *O. clarki* and *O. mykiss* (Table 1). In the case of *S. alpinus*, at least two populations were sampled from each of the three major subspecies proposed by Behnke (1984): *S. alpinus alpinus* from Europe, *S. alpinus erythrinus* from the high Arctic, and *S. alpinus "taranetz"* from the Chukokst-Bering Sea region.

Genomic DNA was extracted from fish livers by phenol extraction (Popodi et al. 1985). The restriction enzymes used in this study included the following enzymes which recognize six base sequences: *Apa*I, *Bam*HI, *Bcl*I, *Bgl*II, *Dra*I, *Eco*RI, *Hind*III, *Kpn*I, *Nhe*I, *Pst*I, *Pvu*II, *Ssp*I, *Sst*I, *Xba*I, and *Xho*I. Restriction enzyme digestions were carried out at 37°C as recommended by the supplier (BRL). Following digestion, samples were separated by electrophoresis for 17 h at 35 V in 0.6% agarose and transferred to a nylon filter (Zeta-bind) as described by Southern (1975). Filters were baked for 2 h at 80°C. Prehybridization was done for 2 h at 65°C in the following solution: 1% BSA, 7% SDS, 500 mM sodium phosphate (pH 7.0), and 1 mM EDTA. Hybridization was done in the same solution overnight at 65°C, and posthybridization washes of filters were carried out at 65°C. Two washes were done for 15 min in 0.5% BSA, 5% SDS, 40 mM sodium phosphate (pH 7.0), and 1 mM EDTA and then two more without the BSA. Filters were sequentially hybridized with radioactive probes to rDNA from mouse and Chinese hamster ovary. Clone I-19 was a 4.2-kb *Eco*RI-*Sal*I fragment containing most of the 28S rDNA coding region from mouse cells and clone PEB-4 was a 1.9-kb *Eco*RI-*Sal*I fragment containing most of the 18S coding region from Chinese hamster ovary cells. Probes were made radioactive

using the random primer reaction according to the manufacturers's instructions (Pharmacia). Filters were dried and exposed to X-Omat film (Kodak) at -70°C with an intensifying screen (DuPont-Cronex).

Genomic DNA from a minimum of three individuals per species was digested singly with each of the 15 enzymes listed above and appropriate double digestions were done to confirm homologies of restriction sites and to construct restriction site maps of the rDNA for each species.

There were two types of variable restriction sites: those which were either present or absent in a given individual (+/- sites) and those which exhibited intraindividual length polymorphisms so that a given individual had a cluster of closely spaced bands spanning a region of a few kilobases (Phillips and Pleyte 1991). Almost all of the sites in the IGS exhibited intraindividual variation, but it was never found for sites in the coding regions or transcribed spacer regions 5'ETS and ITS. In different species, these band clusters were usually either absent or present, but clusters of bands in a couple of different size ranges were found for two enzymes. In the phylogenetic analysis, these sites were considered different between species if the size ranges of the band clusters did not overlap. The +/- sites found in more than one species did not show intraspecific variation with the exception of one site (*Apa*I in the 5'ETS) which was not used in the interspecific phylogenetic analysis. F1 hybrids between *S. fontinalis* and *S. confluentus* had the banding patterns expected for the +/- sites which exhibited differences in the parental species (Phillips and Pleyte 1991).

The restriction site data were analyzed first as discrete characters. Informative sites (those in which two or more taxa contained the restriction site and two or more taxa lacked the restriction site) were identified and phylogenetic trees were constructed using the maximum parsimony method of Swofford's PAUP program (Swofford 1985) for the species in the genus *Salvelinus*, *Salmo*, and *Oncorhynchus* using *H. perryi* as an outgroup. Trees were also obtained for the *Salvelinus* species and *H. perryi* with *Salmo* as an outgroup and for the *Oncorhynchus* species with *Salmo* as an outgroup. The data were bootstrapped using 100 replications.

The proportions of nucleotide differences for each pair of species were also computed. The maximum likelihood estimates of the number of nucleotide differences per site (*d*) between each pair of species were calculated following Nei and Tajima (1983) and divergence estimates between taxa are reported as percent sequence divergence. These data were used to construct distance phenograms using the UPGMA (Sneath and Sokal 1973), neighbor joining (Saitou and Nei 1987), and Fitch-Margoliash (Fitch and Margoliash 1967) (FITCH) methods of the PHYLIP computer program.

TABLE 1. Taxa used in this study and their origin.

Species	No. of fish ^a	Origin
<i>Hucho perryi</i> (Japanese huchen)	2	Hokkaido Fish Hatchery, Japan, 1988
<i>Salvelinus</i>		
<i>S. leucomaenis</i> (Iwana)	6	Hokkaido Fish Hatchery, Japan, 1988
<i>S. fontinalis</i> (brook trout)	4	Dep. Nat. Resour., St. Croix, WI, 1986
	2	Ont. Minist. Nat. Resour., Maple, Ont., 1986
<i>S. namaycush</i> (lake trout)	24	Seneca Lake, NY, 1986
	24	Gull Island Shoals, Bayfield, WI, 1987, 1988
	24	Iron River, MI (Marquette stock), 1987
	24	Jenny Lake, WY, 1987
<i>S. confluentus</i> (bull trout)	4	Arrow Lake, B.C., 1990
	2	Gold Creek, ID, 1990
<i>S. malma</i> (Dolly Varden)	2	Fox River (Kenai), AK, 1988
	3	Auke Creek, AK, 1989
	4	Noatak River, AK, 1990
<i>S. alpinus</i> (Arctic char)	4	Freshwater Inst., MN (Nauyuk Lake, N.W.T.), 1986
	2	Freshwater Inst. (Storvaln, Norway), 1986
	20	Loch Rannoch, Scotland, 1987, 1990
	3	East Finger Lake, AK, 1987
	8	Dolly Varden Lake, AK, 1987
	6	Bangor, ME, 1988
<i>Salmo</i>		
<i>S. trutta</i> (brown trout)	6	Dep. Nat. Resour., St. Croix, WI, 1986
<i>S. salar</i> (Atlantic salmon)	3	River Spey, Scotland, 1987
	3	River Tweed, Scotland, 1987
	4	Grey River, Nfld., 1989
<i>Oncorhynchus</i>		
<i>O. mykiss</i> (rainbow trout)	6	Wisc. Dep. Nat. Resour. (Shasta), 1986
<i>O. clarki</i> (cutthroat trout)	4	Yellowstone Lake, MT, 1990
<i>O. masou</i> (masu salmon)	6	Hokkaido Fish Hatchery, Japan, 1988
<i>O. tshawytscha</i> (chinook salmon)	6	Kitimat River, B.C., 1989
	6	Warm Springs, WA, 1989
<i>O. kisutch</i> (coho salmon)	6	Kewaunee River, WI, 1989
	6	Auke Creek, AK, 1989
<i>O. keta</i> (chum salmon)	6	Yukon River, AK, 1988
	6	Bristol Bay, AK, 1988
<i>O. nerka</i> (sockeye salmon)	2	Cook Inlet, AK, 1988
	2	Bristol Bay, AK, 1989
<i>O. gorbuscha</i> (pink salmon)	12	Mission Creek, B.C., 1990
	6	Prince William Sound, AK, 1987, 1989
	6	Sitka, AK, 1987, 1989
	4	Auke Bay, AK, 1988, 1989
	6	Gastineau Hatchery, Juneau, AK, 1989
	8	Paratunka River, Russia, 1989
	4	Utka River, Russia, 1990

^aThree from each stock were examined for all enzymes, and the additional individuals were examined for enzymes showing polymorphic bands.

Results

Interspecific Variation

Seventy-eight restriction sites in the rDNA were mapped among the 17 species (Fig. 2; Tables 2 and 3). The summary of conserved and variable sites (Table 3) indicates that almost all of the conserved sites were in the coding regions. In Fig. 2 the conserved sites are shown below the map and the phylogenetically informative sites are shown above the map. The 5'ETS and the IGS contained the most informative sites.

A cladistic analysis of the six *Salvelinus* species with the branch and bound option of PAUP using *H. perryi* as the outgroup revealed only one tree of minimal length (Fig. 3). When these data were subjected to bootstrapping, none of the branches was found with greater than 90% confidence limits.

When a similar cladistic analysis was done with the *Oncorhynchus* species using *Salmo* as an outgroup, nine trees were found. The consensus tree is shown in Fig. 4. The trees were similar in overall topology, with the differences occurring in the placement of *O. keta* with respect to the other Pacific salmon and in the placement of *O. masou* with respect to the Pacific trouts and North American Pacific salmon. There were three different topologies for *O. keta* and three different topologies for *O. masou*. *Oncorhynchus masou* was clustered with *O. clarki* and *O. mykiss* in one tree, on a branch between the Pacific trouts and Pacific salmon in another tree, and in a trichotomy with the other two groups in the third tree. In all of the trees, *O. kisutch* and *O. tshawytscha* were placed together and *O. gorbuscha* and *O. nerka* were together. In one of the trees, *O. keta* was placed closer to *O. kisutch* and *O. tshawytscha*, in a second tree, it was placed on a separate branch from the other species, and in the third tree, it was placed exactly in the middle between the two sister groups. When the North American Pacific salmon species were analyzed using *O. masou* as an outgroup, the same trees were obtained for these five taxa. The results of bootstrapping indicated that the branch leading to *O. kisutch* and *O. tshawytscha* was obtained 87% of the time and the branch leading to *O. nerka* and *O. gorbuscha* 63% of the time (Fig. 4). When all 17 taxa were analyzed together with *H. perryi* as the outgroup, *O. masou* clustered with *O. clarki* and *O. mykiss* as shown in the consensus tree (Fig. 5).

Sequence divergence estimates (Tables 4 and 5) were calculated separately for the genera *Salmo* and *Oncorhynchus* and the genera *Salvelinus*, *Salmo*, and *Hucho*: values varied from 0.66% for *S. malma* and *S. alpinus* to 5.74% for *S. namaycush* and *S. trutta* (Table 4) and from 0.98% for *O. kisutch* and *O. tshawytscha* to 5.07% for *O. kisutch* and *S. salar* (Table 5).

The phenograms obtained from the distance data using the Fitch-Margoliash and nearest neighbor methods differed only slightly from the trees obtained from the cladistic analysis of the data. The cladistic analysis of *Salvelinus* placed *S. namaycush* and *S. fontinalis* as sister species, while they were placed on adjacent branches in the phenograms obtained using the distance data. In the cladistic analysis of *Oncorhynchus* the position of *O. keta* was unresolved, but it was clustered with *O. nerka* and *O. gorbuscha* in the phenograms obtained using the distance data. The phenograms obtained from the data using the UPGMA method were very similar. The only differences were that *H. perryi* was clustered with *S. leucomaenis* and *S. confluentus* in the phenogram for the genus *Salvelinus* and that *O. masou* was clustered with

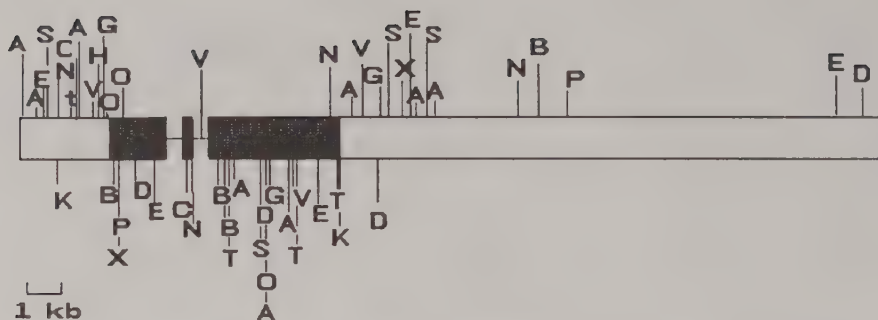


FIG. 2. Restriction map of the rDNA in salmonid fishes showing the sites which are conserved in all 17 species (below the line) and the variable sites which are present in at least two species (informative sites, above the line). Additional sites not shown which were found only in one species from left to right on the map include the following: 5'ETS: ApaI in *S. fontinalis*, HindIII in *S. namaycush*, ApaI and XhoI in *O. tshawytscha*, EcoRI in *O. kisutch*, KpnI in *S. confluentus*, and ApaI in *S. salar*; ITS: DraI in *S. fontinalis*; 28S: KpnI in *S. alpinus* and DraI in *S. malma*; IGS: PstI in *S. salar*, ApaI in *H. perryi*, NheI in *O. keta*, SspI and PvuII in *S. namaycush*, PstI and SstI in *S. trutta*, and BamHI in *S. leucomaenis*. There was also a HindIII site in the IGS just adjacent to the 28S in all of the species except *S. namaycush*.

TABLE 2. Summary of informative sites in the rDNA of salmonid fishes. These informative sites are shown above the map in Figure 2. They are listed in the order in which they appear on the map: sites 1–12 are in the 5' ETS, site 13 is in the 18S, site 14 is in the ITS, site 15 is in the 28S, and sites 16–29 are in the IGS.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
<i>Hucho perryi</i>	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Salvelinus</i>																													
<i>S. namaycush</i>	-	-	-	+	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	+	-	+	+	-
<i>S. fontinalis</i>	-	+	-	+	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-
<i>S. alpinus</i>	-	-	-	+	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-
<i>S. malma</i>	-	-	-	+	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-
<i>S. confluentus</i>	-	-	-	+	-	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. leucomaenis</i>	-	+	-	+	-	+	-	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
<i>Salmo</i>																													
<i>S. trutta</i>	-	-	+	+	-	+	-	-	-	+	+	-	+	-	-	-	+	+	-	+	+	-	-	-	-	+	-	-	+
<i>S. salar</i>	-	-	-	+	-	+	-	-	-	+	+	-	+	-	-	+	+	+	-	+	+	-	-	-	+	-	-	-	+
<i>Oncorhynchus</i>																													
<i>O. masou</i>	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
<i>O. mykiss</i>	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
<i>O. clarki</i>	-	-	+	+	-	-	-	-	+	-	-	+	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+
<i>O. tshawytscha</i>	+	-	+	+	+	+	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>O. kisutch</i>	+	-	+	-	+	+	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>O. keta</i>	+	-	-	+	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>O. nerka</i>	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-
<i>O. gorbuscha</i>	+	-	-	+	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-

TABLE 3. Summary of restriction sites in the rDNA of salmonid fishes.

Subregion	Conserved	Variable	Informative	Total	% informative
5'ETS	1	22	12	22	61
18S	6	1	1	7	14
5.8S	2	0	0	2	0
ITS-1, ITS-2	0	2	1	2	50
28S	15	3	1	18	6
IGS	1	25	14	27	50
Total	25	53	29	78 ^a	38

^aIncludes one 0.5-kb insertion in the 5'ETS.

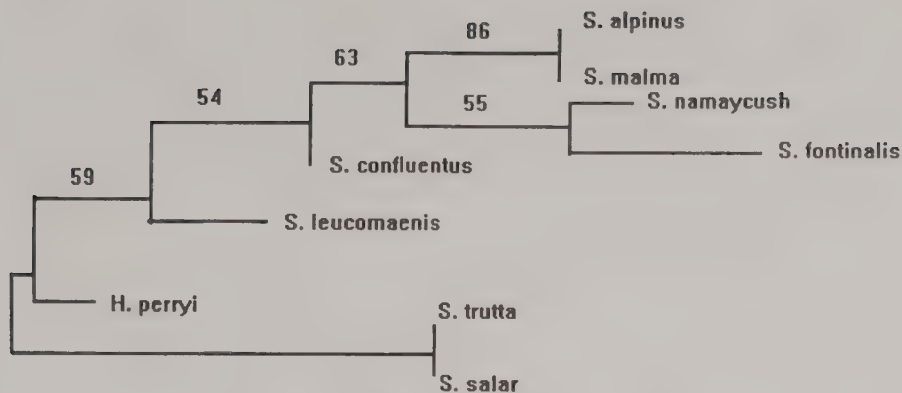


FIG. 3. Single minimum-length tree generated by the PAUP program with the informative sites in the rDNA for the six *Salvelinus* species and *H. perryi* with the two *Salmo* species as an outgroup. Numbers indicate bootstrap values.

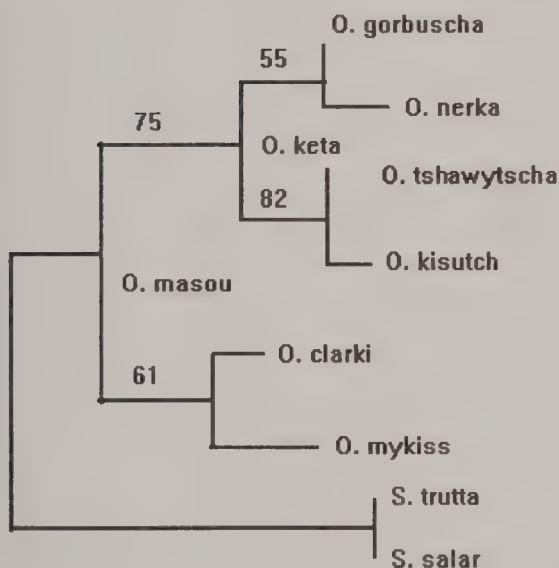


FIG. 4. Consensus tree generated by the PAUP program with the informative sites in the rDNA for the *Oncorhynchus* species with the two *Salmo* species as an outgroup. Numbers indicate bootstrap values.

O. clarki and *O. mykiss* in the phenogram for the genus *Oncorhynchus*.

Intraspecific Variation in *S. alpinus* and *S. malma*

Intraspecific differences in several restriction sites were found among various populations of *S. alpinus* and one difference was found between the various populations of *S. malma* (Table 6). For *S. alpinus*, the three European populations (*S. a. alpinus*) were identical, two populations from the high Arctic (*S. a. erythrinus*) were identical, and the two populations from southern Alaska (*S. a. "taranetz"*) were identical at the sites examined. The two populations of *S. malma lordi* were also identical. When a cladistic analysis was done of the four subspecies of *S. alpinus* and the two subspecies of *S. malma* using *S. namaycush* and *S. fontinalis* as outgroups, 14 different trees were generated. Although *S. a. alpinus* and *S. a. oquassa* were

always on the same branch, the other branches were unresolved with this data.

Discussion

Structure of Ribosomal DNA

The length of the repeating unit of the rDNA in salmonid fishes varies between 22 and 26 kb; most of the length variation occurs within each species (Phillips et al. 1989b; Phillips and Pleyte 1991). In fact, intraindividual variation in total length of the repeating units was very common. The variation in the total length occurs primarily in the IGS and is particularly noticeable in a region 3' to the 28S coding region. The majority of restriction enzymes that cut in this region yield a set of closely spaced bands in each individual. Cloning and sequencing of this region in *S. namaycush* has shown that the length variation is the result of the insertion of variable numbers of a 89-bp repeat in the IGS adjacent to the 28S coding region in different copies of the rDNA within an individual (Zhuo 1991). Some length variation was also observed in the ETS region adjacent to the 18S coding region, but this variation was usually fixed in a given species. The lengths of the ITS-1 and ITS-2 were remarkably conserved in all of the species.

As expected from other studies of rDNA in vertebrates (reviewed in Gerbi 1985; Mindell and Honeycutt 1990) the 5.8S and 18S regions were the most highly conserved, then the 28S coding region, followed by the transcribed spacers, and the IGS. There were 25 sites which were conserved in all species and an additional 27 conserved in *Salvelinus* species. There were 28 conserved sites in *Oncorhynchus* species with one additional site also conserved in the Pacific salmon. A total of 37 sites were conserved in the two species of *Salmo*, providing additional evidence of the close relationship between these two species and the considerable genetic distance between them and the *Oncorhynchus* species.

The highest number of informative sites was found in the 5'ETS. Sites in the 5'ETS are particularly appropriate for the identification of species hybrids. We have shown (Phillips and Pleyte 1991) that F1 hybrids between *S. fontinalis* and *S. confluentus* had the banding patterns expected with the four enzymes which exhibit differences in this region in these two species.

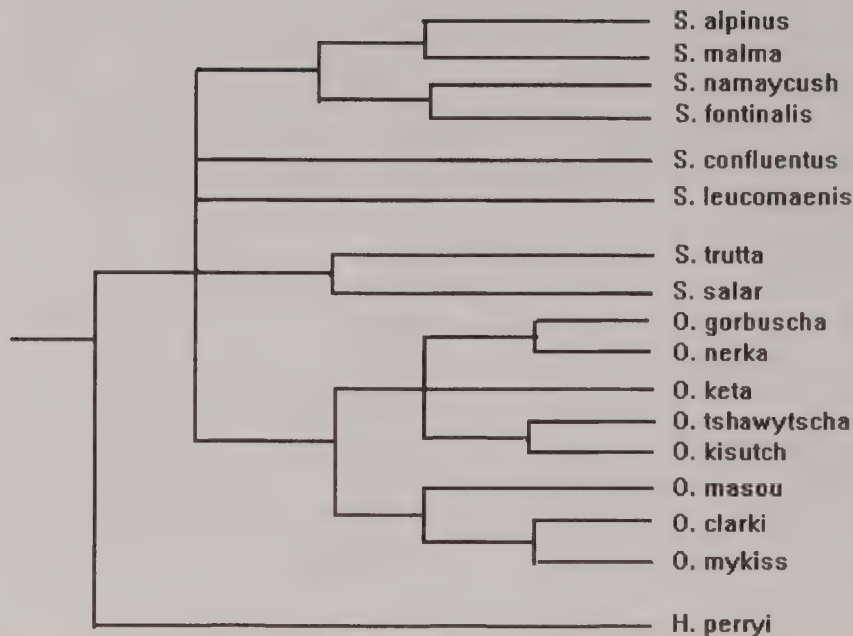


FIG. 5. Strict consensus tree obtained for the 17 species using the maximum parsimony method of the PAUP program and *H. perryi* as an outgroup.

TABLE 4. Matrix of percent sequence divergences (above diagonal) and numbers of shared restriction sites (below diagonal) among rDNA restriction maps for *H. perryi*, six *Salvelinus* species, and two *Salmo* species.

	Huc	leu	con	fon	nam	mal	alp	tru	sal
Huc	0.00	2.81	2.48	3.72	4.56	3.37	3.60	4.06	3.50
leu	30	0.00	2.02	2.64	3.94	3.39	3.61	4.04	4.04
con	28	31	0.00	2.89	3.13	2.02	2.26	3.83	3.83
fon	28	32	29	0.00	2.64	2.64	2.86	5.52	5.52
nam	27	30	29	32	0.00	2.35	2.57	5.74	5.74
mal	29	31	31	32	33	0.00	0.66	4.59	4.59
alp	29	31	31	32	33	37	0.00	4.80	4.79
tru	29	31	29	28	28	30	30	0.00	1.71
sal	30	31	29	28	28	30	30	37	0.00

TABLE 5. Matrix of percent sequence divergences (above diagonal) and numbers of shared restriction sites (below diagonal) among rDNA restriction maps for eight *Oncorhynchus* species and two *Salmo* species.

	tru	sal	clk	myk	mas	tsh	kis	ket	ner	gor
tru	0.00	1.71	3.72	3.83	3.83	3.94	4.50	4.65	4.79	4.65
sal	37	0.00	4.28	3.27	3.27	4.50	5.09	4.65	4.79	4.65
clk	30	29	0.00	1.04	1.59	1.78	2.89	2.40	1.90	1.84
myk	29	30	31	0.00	1.64	2.99	2.99	2.48	1.96	2.48
mas	29	30	30	29	0.00	2.40	2.99	1.90	1.96	1.90
tsh	30	29	31	28	29	0.00	0.98	1.54	2.15	1.54
kis	29	28	29	28	28	33	0.00	2.09	2.15	2.09
ket	28	28	29	28	29	31	30	0.00	1.64	1.04
ner	27	27	29	28	28	29	29	29	0.00	0.53
gor	28	28	30	28	29	31	30	31	31	0.00

Salvelinus Systematics

The major unresolved problems in the genus *Salvelinus* include the relationship of the Asian *S. leucomaenis* to the North American *Salvelinus* and the placement of the North American *S. confluentus* with respect to the other species. In

addition the relationships among various populations and named subspecies of *S. alpinus* and *S. malma* in North America are also unclear.

Because *S. leucomaenis* was not included in previous allozyme and mtDNA surveys, the only published data concerning its relationships to other species have been from morphological

TABLE 6. Variable restriction sites in the rDNA of *S. alpinus* and *S. malma*.

Population	ApaI (ETS)	KpnI (28S)	SspI (1GS)	DraI (28S)	DraI (1GS)	N
<i>S. alpinus</i> ,						
<i>s. a. alpinus</i>						
Storvaln, Norway	—	+	+	—	+	6
Lake Rannoch, Scotland	—	+	+	—	+	12
<i>S. a. oquassa</i>						
Bangor, ME	—	+	—	—	—	10
<i>S. a. erythrinus</i>						
Nayuk Lake, N.W.T.	+	—	—	—	—	10
<i>S. a. "tarentetz"</i>						
Dolly Varden Lake, AK	—	—	—	—	—	10
East Finger Lake, AK	—	—	—	—	—	6
<i>S. malma</i> ,						
<i>S. m. lordi</i>						
Auke Creek, Ak	+	—	—	+	—	4
Fox River (Kenai), AK	+	—	—	+	—	4
<i>S. m. malma</i>						
Noatak River, AK	—	—	—	+	—	4

comparisons (Behnke 1980; Cavender 1980; Stearley 1990) and karyological comparisons (Cavender 1984; Cavender and Kimura 1989; Phillips et al. 1989a, 1989b). Behnke (1980) suggested that *S. leucomaenis* was more closely related to *S. alpinus* and *S. malma* than to *S. namaycush* and *S. fontinalis* and placed these three species with *S. confluentus* in the subgenus *Salvelinus*. Both he and Cavender concluded that *S. leucomaenis* shares a number of characters with *S. confluentus* which is very similar to *S. albus* found in Kamchatcha. In two cladograms of species in the genus *Salvelinus* (Cavender and Kimura 1989), one based on morphological characters and the other on karyological characters, *S. namaycush* and *S. fontinalis* are on the first branch after *Hucho*, then *S. leucomaenis* and *S. confluentus* on a second branch, and finally *S. malma* and *S. alpinus*. However, Stearley's (1990) tree based on osteological data did not support this topology. The conclusion from the rDNA restriction site data is that *S. leucomaenis* is more closely related to *S. confluentus* than to the other *Salvelinus* species. This is also supported by a recent allozyme survey (Crane 1991) and by analysis of sequence data from a portion of the ITS-1 of the rDNA (Pleyte 1991).

It should be pointed out that the branching pattern of the species in the genus *Salvelinus* obtained from the rDNA restriction site data analysis is not congruent with distance data from both rDNA and mtDNA which suggest a close relationship between *S. confluentus* and *S. malma* and *S. alpinus*. There are several possible explanations for this. First, the branching pattern obtained from the rDNA data may not be correct, since the confidence limits are less than 90% as shown by the bootstrapping results. Second, the branching pattern could be correct but the evolutionary rates might not be equal along the two branches. If the branch leading to *S. fontinalis* and *S. namaycush* is longer than the other branches, *S. confluentus* could be genetically closer to *S. malma* and *S. alpinus*, but still be lower on the cladogram than *S. fontinalis* and *S. namaycush*. Third, this could be the result of hybridization of *S. confluentus* with either *S. malma* or *S. alpinus* after the lineages had diverged. This might explain the closer genetic distance obtained between *S. confluentus* and the *S. malma* - *S. alpinus* complex with the mtDNA data, compared with the nuclear rDNA data. *Salvelinus confluentus* clustered within the

S. alpinus subgroup in the tree based on mtDNA data. The number of informative sites obtained from both the rDNA and mtDNA restriction site maps was rather small, so that additional data from sequencing these molecules will be needed to resolve these relationships. Preliminary analysis of the sequence of part of the ITS-1 of the rDNA (Pleyte 1991) supported a close relationship between *S. confluentus* and *S. leucomaenis*, but the branching patterns among the six species were not completely resolved. We expect that complete sequencing of the ITS regions will yield sufficient data to resolve the relationships among the taxa in the genus *Salvelinus*.

Relationships among the various subspecies of *S. alpinus* and *S. malma* also remain controversial: Savvaitova (1980) suggested that they all belong to a single species. On the basis of morphology, Behnke (1984) suggested that there are three major groups of Arctic char with origins in Europe (*S. a. alpinus*), Siberia and the high Arctic region of North America (*S. a. erythrinus*), and a third form found in the Asian and North American drainages of the Chukotsk and Bering seas (*S. a. "tarentetz"*). The landlocked form in Maine (*S. a. oquassa*) is thought to be most closely related to the European form. Our data support Behnke's interpretation: each of these types has a different pattern of restriction sites, and the Maine form is most closely related to the European form (see Table 6). Behnke (1984) also assigned subspecific status to the northern and southern forms of *S. malma*. Both of these forms of *S. malma* were closest to the *S. a. erythrinus* subspecies of *S. alpinus*, but these groups were not well resolved with the limited data available.

Oncorhynchus Systematics

Problems also remain in the systematics of *Oncorhynchus*. The recent assignment of the Pacific trout with the Pacific salmon to the genus *Oncorhynchus* is based on many types of data (reviewed in Smith and Stearley 1989), and our results support this relationship. However, the relationships of the various Pacific trouts to each other and the Pacific salmon are still unclear. The trees based on rDNA data agree with those based on allozyme data (Utter et al. 1973) in placing *O. masou* close to the North American Pacific trouts (*O. mykiss* and *O. clarki*). The rDNA data did not support the tree obtained with mtDNA data in which these three species are clustered with *O. kisutch*

and *O. tshawytscha* (Thomas et al. 1986; Ankenbrandt 1988). Questions also remain concerning the relationships among the three advanced Pacific salmon species. Trees based on allozyme and morphological traits place *O. gorbuscha* and *S. nerka* as sister species, while mtDNA and life history traits place *O. keta* and *O. gorbuscha* as sister species (reviewed in Smith and Stearley 1989). These two species also share a common t-RNA derived retroposon (Kido et al. 1991). The relationship between *O. keta* and the other two advanced Pacific salmon is unresolved by the rDNA restriction site data, but there was no support for a sister relationship between *O. keta* and *O. gorbuscha*. The congruence of data from morphology, allozymes, and nuclear rDNA on the one hand and life history and mtDNA on the other hand suggests that transfer of mtDNA following hybridization between diverged lines may have occurred. This possibility is also supported by the conflict between estimates of evolutionary divergence times between *O. keta* and *O. gorbuscha* of greater than 5–7 million yr based on fossil evidence (Smith et al. 1982) and estimates of divergence times of 1.25 million yr based on mtDNA divergence (Thomas et al. 1986). However, this assumes a rate of 2% substitution per million years per pair of lineages derived from mtDNA of mammals, and evidence is accumulating (Bentzen 1988) that rates are slower in teleosts. Only a few informative sites for these species were obtained in our analysis of rDNA, so that sequence data will be needed to help resolve the relationships between these species.

Our restriction site analysis has shown that the various spacer regions of the rDNA are evolving at rates appropriate for phylogenetic comparisons among species and genera of salmonid fishes. However, restriction site data have disadvantages for phylogenetic analysis. Site gains are more probable than site losses, and the accuracy of mapping is plus or minus 300 bp. The large size of the IGS, the numerous insertions found there, and the lack of a homologous probe to this region resulted in an incomplete mapping of this region. DNA sequence data would be the most desirable for phylogenetic analysis. The most informative sites were found in the 5'ETS. We are sequencing clones of this region from *S. namaycush* so that primers can be prepared for amplification and direct sequencing of the 5'ETS in different species. Regions of the rDNA which have inter-specific variation, but very little intraspecific variation, would be the most desirable for sequencing. One such region is the ITS-1 which was used in a phylogenetic analysis of the great apes (Gonzales et al. 1990). We have designed primers for amplification of the ITS-1 and ITS-2 by the polymerase chain reaction and are in the process of accumulating sequence data on these regions that we hope will help resolve some of the unanswered questions in the systematics of this most interesting group of fishes.

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with the analysis of data. Restriction maps for any of the species are available on request.

References

- ANKENBRANDT, L. G. 1988. The phylogenetic relationships of the Pacific fishes contained in the teleost genera *Oncorhynchus* and *Salmo* based on restriction fragment analysis of mitochondrial DNA. M.S. thesis, University of Washington, Seattle, WA.
- BALON, E. K. 1980. Comparative ontogeny of charrs, p. 703–720. In E. K. Balon [ed.] Charrs, salmonid fishes of the genus *Salvelinus*. Vol. 1. Dr. W. Junk Publishers, The Hague, The Netherlands.
- BEHNKE, R. J. 1965. A systematic study of the family Salmonidae with special reference to the genus *Salmo*. Doctoral dissertation, University of California-Berkeley, Berkeley, CA.
1980. A systematic review of the genus *Salvelinus*, Chap. 14, p. 441–481. In E. K. Balon [ed.] Charrs, salmonid fishes of the genus *Salvelinus*. Vol. 1. Dr. W. Junk Publishers, The Hague, The Netherlands.
1984. Organizing the diversity of the Arctic char complex. In L. Johnson, McV. Clarke, and K. E. Marshall [ed.] Biology of the Arctic charr. University of Manitoba Press, Winnipeg, Man.
1989. Interpreting the phylogeny of *Salvelinus*. *Physiol. Ecol. Jpn. Spec.* Vol. 1: 35–48.
- BENTZEN, P. 1988. Mitochondrial DNA polymorphism in American shad (*Alosa sapidissima*) and its implications for population structure. Ph.D. thesis, McGill University, Montreal, Que. 119 p.
- CAVENDER, T. M. 1978. Taxonomy and distribution of the bull trout, *Salvelinus confluentus* (Suckley) from the American Northwest. *Calif. Fish Game* 64: 139–174.
1980. Systematics of *Salvelinus* from the North Pacific Basin, Chap. 6, p. 295–322. In E. K. Balon [ed.] Charrs, salmonid fishes of the genus *Salvelinus*. Vol. 1. Dr. W. Junk Publishers, The Hague, The Netherlands.
1984. Cytotaxonomy of North American *Salvelinus*, p. 431–445. In L. Johnson and B. L. Burns [ed.] Biology of the Arctic charr. Proc. of Int. Symp. on Arctic Charr, Winnipeg, Manitoba, May 1981. University of Manitoba Press, Winnipeg, Man.
- CAVENDER, T. M., AND S. KIMURA. 1989. Cytotaxonomy and interrelationships of Pacific basin *Salvelinus*. *Physiol. Ecol. Jpn. Spec.* Vol. 1: 49–68.
- COEN, E., T. STRACHAN, AND G. DOVER. 1982. Dynamics of concerted evolution in regions of ribosomal DNA and histone gene families in the melanogaster group of *Drosophila*. *J. Mol. Biol.* 158: 17–35.
- CRANE, P. A. 1991. Biochemical systematics of the salmonid genus *Salvelinus*. M.S. thesis, Southern Illinois University, Carbondale, IL.
- FERRIS, S. D., R. D. SAGE, C. M. HUAN, J. N. NIELSEN, U. RITTE, ET AL. 1983. Flow of mitochondrial DNA across a species boundary. *Proc. Natl. Acad. Sci. USA* 80: 2290–2294.
- FITCH, W. M., AND E. MARGOLIASH. 1967. Construction of phylogenetic trees. *Science (Wash., DC)* 157: 279–284.
- GERBI, S. A. 1985. Evolution of ribosomal DNA, p. 419–517. In R. J. MacIntyre [ed.] Molecular evolutionary genetics. Plenum Press, New York, NY.
- GINATULINA, L. K., S. V. SHEDKO, I. L. MIROSHNICHENKO, AND A. A. GINATULIN. 1988. Sequence divergence in mitochondrial DNA from the Pacific salmon. *Zh. Evol. Biokhim. Fiziol.* 24: 477–483.
- GONZALES, I. L., J. E. SYLVESTER, T. F. SMITH, D. STAMBOLIAN, AND R. D. SCHMICKEL. 1990. Ribosomal RNA gene sequences and hominoid phylogeny. *Mol. Biol. Evol.* 7: 203–219.
- GREWE, P. M., N. BILLINGTON, AND P. D. N. HEBERT. 1990. Phylogenetic relationships among members of *Salvelinus* inferred from mitochondrial DNA divergence. *Can. J. Fish. Aquat. Sci.* 47: 984–991.
- GYLLENSTEN, U., AND A. C. WILSON. 1987. Mitochondrial DNA of salmonids: inter- and intraspecific variability detected with restriction enzymes, p. 301–307. In N. Ryman and F. Utter [ed.] Population genetics and its application to fisheries management. University of Washington Press, Seattle, WA.
- HARTLEY, S. 1987. The chromosomes of salmonid fishes. *Biol. Rev.* 62: 197–214.
- HILLIS, D. M., AND S. K. DAVIS. 1986. Evolution of ribosomal DNA: fifty million years of recorded history in the frog genus *Rana*. *Evolution* 40: 1275–1288.
- JOHNSON, K. R. 1984. Protein variation in the salmonidae: genetic interpretations of electrophoretic banding patterns, linkage associations among loci, and evolutionary relationships among species. Ph.D. thesis, Pennsylvania State University, University Park, PA.
- KENDALL, A. W. JR., AND R. J. BEHNKE. 1984. Salmonidae: development and relationships, p. 142–149. In H. G. Moser [ed.] Ontogeny and system-

- atics of fishes. Spec. Publ. 1. American Society of Ichthyologists and Herpetologists, Gainesville, FL.
- KIDO, Y., M. AONO, T. YAMAKI, K. MATSUMOTO, S. MURATA, M. SANEYOSHI, AND N. OKADA. 1991. Shaping and reshaping of salmonid genomes by amplification of tRNA-derived retroposons during evolution. *Proc. Natl. Acad. Sci. USA* 88: 2326–2330.
- MINDELL, D. P., AND R. L. HONEYCUTT. 1990. Ribosomal RNA in vertebrates: evolution and phylogenetic applications. *Annu. Rev. Ecol. Syst.* 21: 541–556.
- NEI, M., AND F. TAJIMA. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction site data. *Genetics* 105: 207–217.
- NORDEN, C. R. 1961. Comparative osteology of representative salmonid fishes, with particular reference to the grayling (*Thymallus arcticus*) and its phylogeny. *J. Fish. Res. Board Can.* 18: 679–791.
- PAVLOV, D. A. 1980. Peculiarities of the embryonic and larval development of Atlantic and Pacific salmon of the genus *Salmo* with respect to their evolution. *Zool. Zh.* 59: 569–576.
- PHILLIPS, R. B., AND K. A. PLEYTE. 1991. Nuclear DNA and salmonid phylogenetics. *J. Fish Biol.* 39S: 259–275.
- PHILLIPS, R. B., K. A. PLEYTE, AND P. E. IHSEN. 1989a. Patterns of chromosomal nucleolar organizer (NOR) variation in fishes of the genus *Salvelinus*. *Copeia* 1989: 47–53.
- PHILLIPS, R. B., L. M. VAN ERT, AND K. A. PLEYTE. 1989b. Evolution of nucleolar organizer regions (NORs) and rDNA in fishes of the genus *Salvelinus*. *Physiol. Ecol. Jpn. Spec. Vol.* 1: 429–447.
- PLEYTE, K. A. 1991. A phylogenetic analysis using ribosomal DNA internal transcribed spacer sequences of the genus *Salvelinus*. Ph.D. thesis, University of Wisconsin-Milwaukee, Milwaukee, WI.
- POPODI, E. M., D. GREVE, R. B. PHILLIPS, AND P. J. WEJESNORA. 1985. The ribosomal RNA genes in three salmonid species. *Biochem. Genet.* 23: 997–1010.
- SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406–425.
- SAVVAITOVA, K. A. 1980. Taxonomy and biogeography of charrs in the Palearctic, Chap. 5, p. 281–294. *In* E. K. Balon [ed.] *Charrrs, salmonid fishes of the genus Salvelinus*. Vol. 1. Dr. W. Junk Publishers, The Hague, The Netherlands.
- SMITH, G. R., AND R. F. STEARLEY. 1989. The classification and scientific names of rainbow and cutthroat trout. *Fisheries* 14: 4–10.
- SMITH, G. R., K. SWIRYDCZUK, P. G. KIMMEL, AND B. H. WILKINSON. 1982. Fish biostratigraphy of late Miocene to Pleistocene sediments of the western snake river plain, Idaho. *In* B. Bonnicksen and R. M. Breckenridge [ed.] *Cenozoic geology of Idaho*. Idaho Bur. Mines Geol. Bull. 26: 519–541.
- SNEATH, P. H. A., AND R. R. SOKAL. 1973. *Numerical taxonomy*. W. H. Freeman, San Francisco, CA.
- SOUTHERN, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98: 503–517.
- STEARLEY, R. F. 1990. *Historical biology of Oncorhynchus*. University of Michigan, Ann Arbor, MI.
- SWOFFORD, D. L. 1985. *PAUP: Phylogenetic analysis using parsimony*. Illinois Natural History Survey, Champaign, IL.
- THOMAS, W. K., R. E. WITHLER, AND A. T. BECKENBACH. 1986. Mitochondrial DNA analysis of Pacific salmonid evolution. *Can. J. Zool.* 64: 1058–1064.
- TSUYUKI, H., AND E. ROBERTS. 1966. Inter-species relationships within the genus *Oncorhynchus* based on biochemical systematics. *J. Fish. Res. Board Can.* 23: 101–107.
- UTTER, F. M., F. W. ALLENDORF, AND H. O. HODGINS. 1973. Genetic variability and relationships in Pacific salmon and related trout based on protein variations. *Syst. Zool.* 22: 257–270.
- WILSON, G. N., M. KNOLLER, L. L. SZYURA, AND R. D. SCHMICEK. 1984. Individual and evolutionary variation of primate ribosomal DNA transcription initiation regions. *Mol. Biol. Evol.* 1: 221–237.
- ZHUO, L. 1991. Molecular and cytogenetic analysis of ribosomal DNA variants in lake trout (*Salvelinus namaycush*). Ph.D. thesis, University of Wisconsin-Milwaukee, Milwaukee, WI.

Response of Amphipoda and Trichoptera to Lake Fertilization in the Canadian Arctic

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Jorgenson, J. K., H. E. Welch, and M. F. Curtis. 1992. Response of Amphipoda and Trichoptera to lake fertilization in the Canadian Arctic. Can. J. Fish. Aquat. Sci. 49: 2354–2362.

Small oligotrophic lakes at Saqvaqujac, Northwest Territories, were fertilized with phosphorus and nitrogen after 2 yr of study and the response of macroinvertebrates to increased primary production was followed for 3 yr. The amphipod *Gammarus lacustris lacustris* (G. O. Sars) had a 2-yr life cycle, with three cohorts present in August. Biomass under natural conditions was approximately 0.1–0.2 g dry wt·m⁻². *Gammarus* responded immediately to a doubling of phytoplankton production with increased survival of young-of-year. *Gammarus* biomass increased steadily to 0.9 g dry wt·m⁻² and had not stabilized after 3 yr of fertilization. Trichoptera were represented by three species, with *Grensia praeterita* composing the bulk of the biomass, followed by *Apatania zonella* and an uncommon *Hesperophylax* species. Trichoptera biomass ranged from 0.04 to 0.3 g dry wt·m⁻² before fertilization. Response to increased primary production was slow, beginning in year 2 of fertilization. Trichoptera biomass had doubled by the third year of fertilization but was probably several years from equilibrium. Application of benthos models, in addition to the data, suggested that the production to biomass ratio was between 1 and 2, averaging 1.5.

À Saqvaqujac (Territoires du Nord-Ouest), des petits lacs oligotrophes ont été fertilisés au phosphore et à l'azote après 2 ans d'étude et la réaction des macroinvertébrés à la production primaire accrue a été suivie pendant 3 ans. L'amphipode *Gammarus lacustris lacustris* (G. O. Sars) avait un cycle biologique de 2 ans, avec trois cohortes présentes en août. Dans les conditions naturelles, la biomasse était d'environ 0,1 à 0,2 g poids sec·m⁻². *Gammarus* a réagi immédiatement à un doublement de la production de phytoplancton par un taux de survie accru des jeunes de l'année. La biomasse de *Gammarus* a augmenté régulièrement à 0,9 g poids sec·m⁻² et ne s'était toujours pas stabilisée 3 ans après la fertilisation. Les trichoptères étaient représentés par trois espèces, *Grensia praeterita* constituant la majeure partie de la biomasse suivie de *Apatania zonella* et d'une espèce rare de *Hesperophylax*. La biomasse des trichoptères variait de 0,04 à 0,3 g poids sec·m⁻² avant la fertilisation. La réaction à la production primaire accrue a été lente et a commencé au cours de la deuxième année de la fertilisation. La biomasse des trichoptères avait doublé à la troisième année de la fertilisation, mais se situait probablement à plusieurs années de l'équilibre. L'application de modèles du benthos, en plus des données, semble indiquer que le rapport production – biomasse est compris entre 1 un 2 et est égal en moyenne à 1,5.

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Whole-lake fertilization experiments were performed at Saqvaqujac, in the Canadian central Arctic (Fig. 1), from 1978 through 1983 (see Welch 1985 for background information). One objective was to determine if arctic lakes would respond to fertilization the same way that temperate zone lakes do, as shown by the small artificially eutrophied Precambrian Shield lakes at the Experimental Lakes Area in northwestern Ontario (Schindler 1980). A second objective was to determine the time course of any increase in productivity through the food chain, from photosynthesis to fish growth.

The benthos of small oligotrophic lakes at Saqvaqujac is dominated by chironomids (Diptera: Chironomidae). Caddisflies (Trichoptera) of several genera are usually present, and the amphipod *Gammarus lacustris lacustris* (G. O. Sars) is abundant in most of the small (<10 ha) and all of the larger lakes. A few adult stoneflies (Plecoptera) emerge at the beginning of ice-out, and fingernail clams (Sphaeriidae) are common. The fish (landlocked Arctic char (*Salvelinus alpinus*) and lake trout (*S. namaycush*)) depend primarily on Chironomidae,

Amphipoda, and Trichoptera for food. Because we were under severe restraints as to the resources available for this project, we opted to study only the major groups of benthos, using techniques that we hoped would document abundance and possibly production changes before and after fertilization. Thus, for chironomids we quantified emergence (the production of adults), which increased in the same year that primary production increased, and stabilized at the higher level by the second year of fertilization (Welch et al. 1988).

We opted to quantify Trichoptera larvae and pupae, and amphipods, by intensive sampling annually during the open-water season only. The Trichoptera are represented by three common species of the family Limnephilidae. The most abundant is *Grensia praeterita* (Walker), a common species throughout the Canadian Arctic (Nimmo 1986). *Apatania zonella* (Zetterstedt) is a conspicuous component of the shallow-water benthos of circumpolar high-arctic lakes (e.g. Char Lake, Cornwallis Island (75°N), and Lake Hazen, Ellesmere Island (82°N)) and occurs widely in the Canadian Arctic (Nimmo 1986). Last, a *Hesperophylax* species is present in the four experimental lakes but is not abundant. *Apatania* is a

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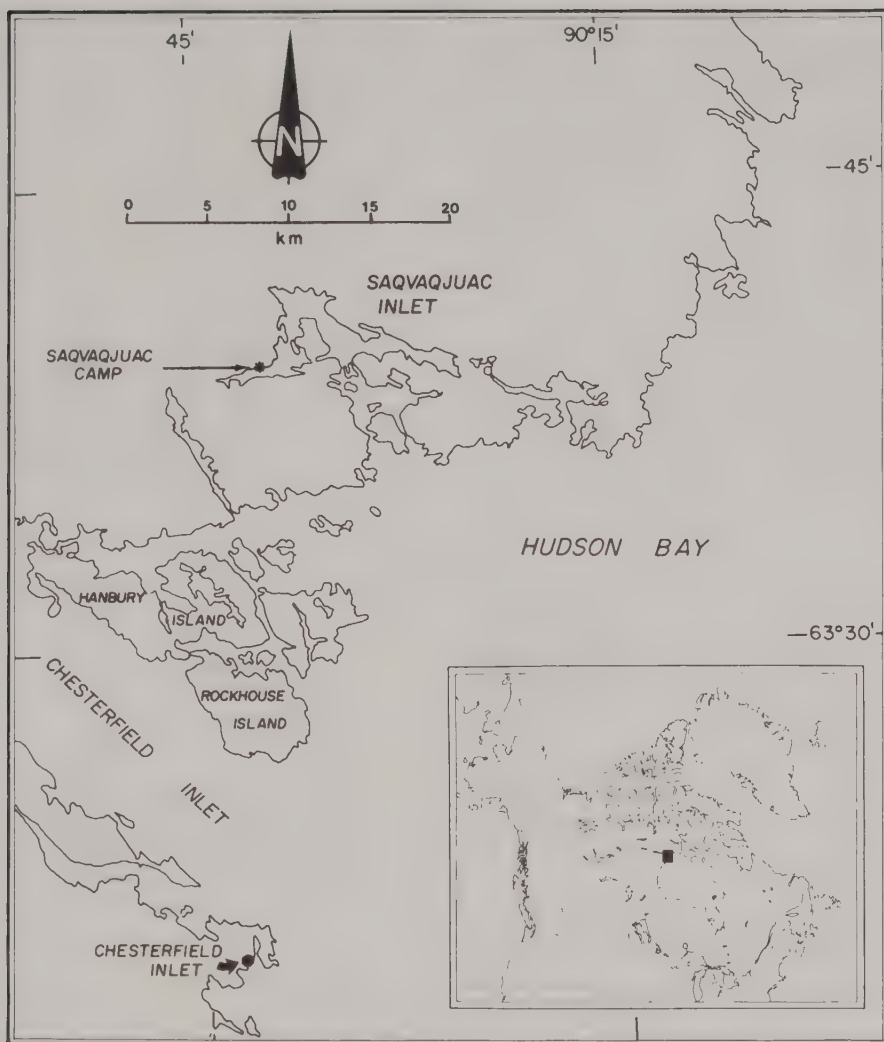


FIG. 1. Location of the Saqvaquac Field Station, Department of Fisheries and Oceans. The four experimental lakes discussed in this paper are all within several kilometres of the camp.

TABLE 1. Data for the experimental Saqvaquac lakes.

Lake	Surface area (ha)	Z_{mean} (m)	Z_{max} (m)	Mean annual temperature ($^{\circ}\text{C}$)	% ice volume ^a	Years nutrients added ($\text{g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$)
Jade	3.6	1.8	5.2	3.8	77	1979–80 (0.2 g P, 2.0 g N)
Far	3.7	3.6	8.9	3.7	46	1979–83 (0.1 g P)
P&N	7.1	3.3	10.2	3.4	47	1979–83 (0.1 g P, 1.0 g N)
Spring	6.9	2.7	7.1	4.0	56	

^aAt 2 m ice thickness.

scraper and *Grensia* and *Hesperophylax* are shredder/detritivores (Wiggins 1977). All probably graze benthic detritus and algae for food in Saqvaquac lakes.

Methods

Study Area and Experimental Regime

The experimental site was near Saqvaquac Inlet, an enclosed arm of Hudson Bay north of Chesterfield Inlet (Fig. 1). Bedrock

is Precambrian granitic gneiss, exposed on hills of 10–30 m relief. Valleys are partly filled with coarse glacial till and are usually occupied by lakes of various sizes which cover 17% of the Saqvaquac area (Welch 1985). A thin layer of epilithic benthic algae in the littoral zone contributes a large portion of lakewide primary production in the experimental lakes (H. E. Welch, unpubl. data). The lakes freeze about 20 September and become ice-free between 1 and 20 July.

The littoral zone of each lake, frozen to a depth of about 2 m each winter (Welch et al. 1987), is mostly composed of gravel

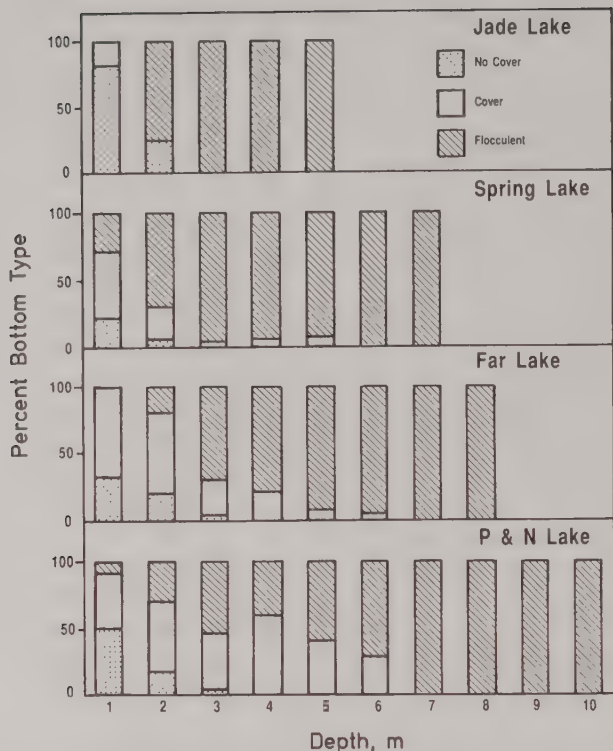


FIG. 2. Bottom substrate types in the experimental lakes at Saqvaqujac. No cover: large boulders, bedrock, sand, or hard-packed clay; cover: frost-shattered rock, gravel, or substrate with macrophytes (rare), overlain by less than 2 cm of flocculent sediment; flocculent: fine sediment thicker than 2 cm.

and rock, with some exposed bedrock. Deeper, this rubble gives way to a flocculent silt-clay sediment which covers the deepest parts of all the lakes (Fig. 2).

After 2 yr of background data collection (1977–78), Far Lake received $0.1 \text{ g P} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ (as H_3PO_4) in weekly installments during the ice-free season 1979–83 (Table 1). P&N Lake received 0.1 g P and 1.0 g N (as NaNO_3) at the same time. Jade Lake, which was not studied prior to fertilization, received 0.2 g P and 2.0 g N in 1979 and 1980, and nothing after that. Spring Lake served as an unfertilized control 1977–83. Phytoplankton production in P&N Lake doubled from 15 g C before fertilization to $30 \text{ g C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ after fertilization with phosphorus and nitrogen (Welch et al. 1989). Turbidity increased sharply in Jade and P&N lakes during fertilization, as a result of increased phytoplankton biomass. There were no significant changes in phytoplankton production in Spring and Far lakes during the study (Welch et al. 1989). Welch (1985) gives more information on site characteristics and fertilization schedule.

Sampling

Quadrat sampling was done by the same diver (J.J.) at pre-determined stations along permanent transects. A pilot study done in 1979 (unpubl. data) indicated that a quadrat size of 0.25 m^2 was optimal. All loose material inside the quadrat was removed by suction hose to the surface and into an acrylic vacuum box holding a $200\text{-}\mu\text{m}$ net. The sample was washed through the net, rinsed into jars, and preserved in 10% for-

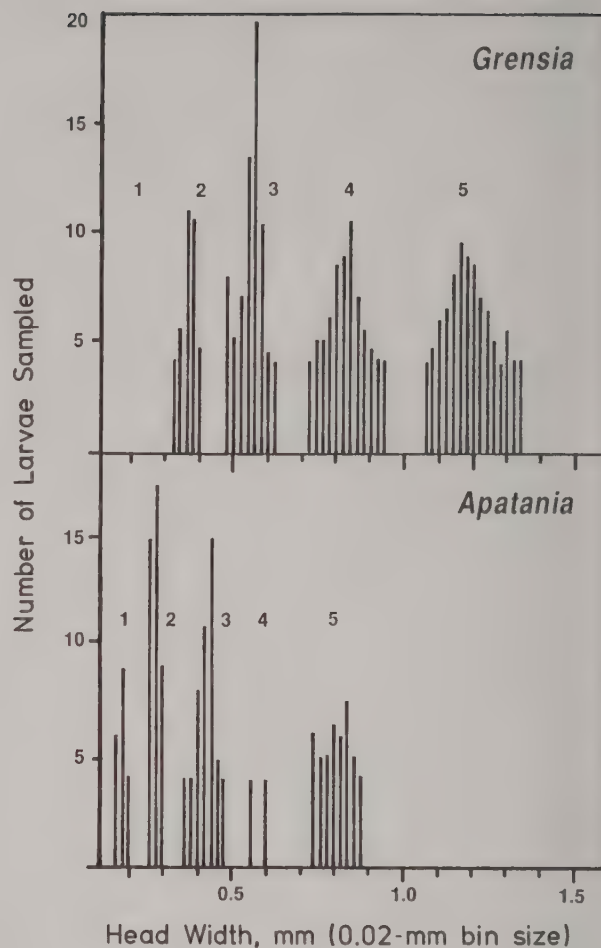


FIG. 3. Frequency distribution of *Gressia* and *Apatania* head width.

malin. The diver classified the substrate sampled as one of three types: cover, no cover, and flocculent (legend, Fig. 2).

Sixty quadrats each were sampled annually in August from P&N and Spring lakes and 50 quadrats in Far and Jade lakes. Far Lake had 12 transects, Jade Lake had 10, and P&N and Spring lakes each had 16. Samples were weighted by 1-m strata such that sampling equally covered all depths and areas of each lake.

Amphipods were sexed using gnathopod morphology. The presence of bristles (setae) on oostegites to form a brood pouch defined sexual maturity for females. Head length was measured to the nearest 0.02 mm to allow the calculation of year class (cohort) abundance. *Gammarus* less than 0.7 mm head length were not sexable and were termed young-of-year (YOY).

Trichoptera were identified to one of the three species present and head width measured to the nearest 0.02 mm . In common with most Trichoptera, the three species each had five larval instars. The mean increase in larval head width between instars for both *Apatania* and *Gressia* (for which there were the most data) was 1.45 mm for all lakes and years (range $1.40\text{--}1.57 \text{ mm}$ for a given lake, year, and genus), calculated from the antilog of the slope of $\ln(\text{mean instar size})$ versus instar. The length-frequency distributions were generally nonoverlapping between instars for each genus, so that larvae could accurately be assigned to instar and genus (Fig. 3). The only exceptions were

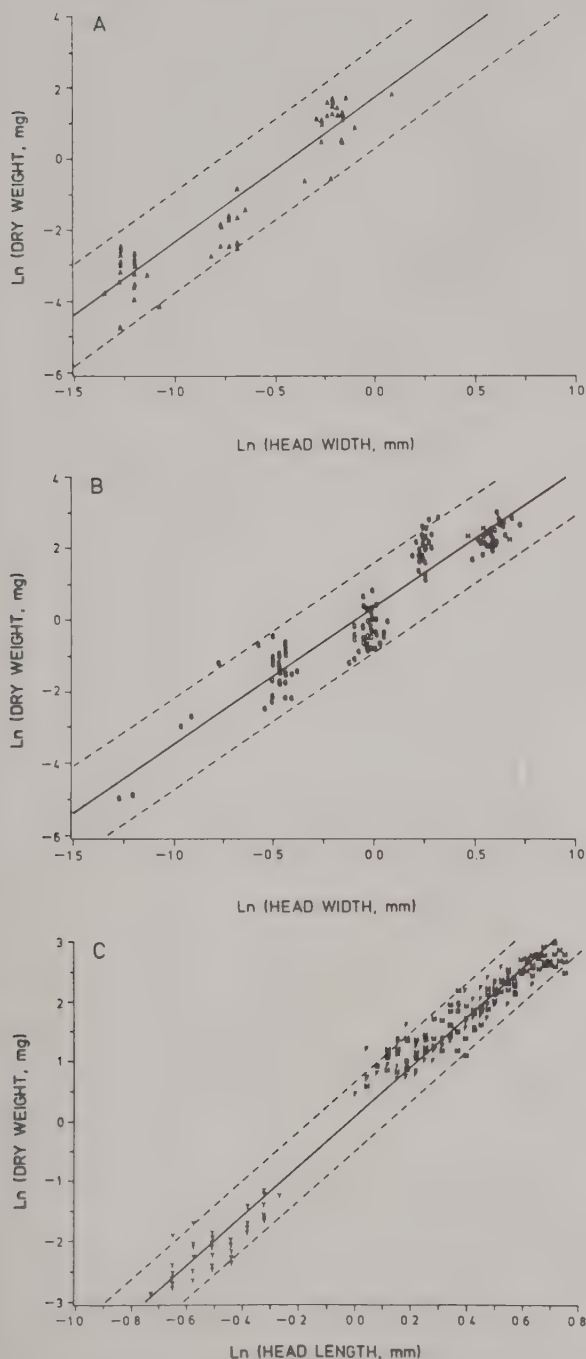


FIG. 4. Head size – dry weight relationships for Trichoptera and *Gammarus lacustris* at Saqvaquac. Broken lines enclose 95% confidence limits for individual points. (A) *A = Apatania zonella*: $\ln \text{ dry weight} = 4.102 \ln \text{ head width} + 1.760$. (B) *H = Hesperophylax praeterita*, *G = Grensia* sp.: $\ln \text{ dry weight} = 3.856 \ln \text{ head width} + 0.396$; approximate fifth-instar dry weight is 12 mg for *Grensia* and *Hesperophylax* and 4.5 mg for *Apatania*. (C) *Gammarus lacustris* (Y = young-of-year, M = male, F = female (immatures and adults)): $\ln \text{ dry weight} = 4.130 \ln \text{ head length} + 0.097$; approximate adult dry weight is 12 mg.

for first-instar *Grensia* and second-instar *Apatania*, which could not be distinguished either taxonomically or by size. Most were probably *Grensia* because that genus was 10 times more abundant than *Apatania*.

Head sizes and dry weights were determined on freshly collected Amphipoda and Trichoptera. Analysis of covariance on $\ln$ (total dry weight of fresh animals) versus $\ln$ (head size of preserved animals) with sex (amphipods) or genus (Trichoptera) as the treatment indicated no differences between male and female amphipods, so they were combined. However, there were significantly different intercepts among Trichoptera genera. Pairwise analysis of combinations of genera indicated that *Grensia* and *Hesperophylax* were similar and could be combined. The resulting regression coefficients (Fig. 4) were used for conversion of head size to weight and the calculation of biomass.

Numbers and biomass per square metre for Amphipoda and Trichoptera were derived as follows. The mean number per square metre for each depth stratum was calculated and multiplied by the stratum area to obtain a value for each depth stratum. The total of those was then divided by the lake area to obtain mean numbers per square metre of lake surface.

The estimate of mean density over all strata had to take into account unequal stratum variance. Transformation techniques are often used to reduce such heteroscedasticity (Steele and Torrie 1980) but we avoided this because bias of the transformation and subsequent back-transformation due to nonsymmetry of the frequency distribution was determined to be a greater problem than lack of equality of variance. Rather, we used the following method to obtain an overall variance to use for statistics such as confidence limits.

The variable of interest was $\bar{D}$, the estimated average mean density over all depth strata for each lake/year. We required an expression for its variance, $\text{Var}(\bar{D})$, from which to compute its standard error, $\sqrt{\text{Var}}$.

To get $\bar{D}$ given the stratum mean (X_i) and standard deviation (S_i), the stratum sample size (N_i), and the stratum area (A_i) in stratum i ($i = 1, 2, \dots, n$):

$$\bar{D} = \sum P_i \bar{X}_i$$

where $P_i = A_i / \sum A_i$, the proportion of lake in stratum i .

$$\text{Var}(\bar{D}) = \sum (P_i^2 \text{Var}(\bar{X}_i))$$

$$\text{Var}(\bar{X}_i) = s_i^2 / N_i.$$

Taylor's Power Law states

$$\sigma^2 = \alpha \mu^b$$

and

$$s_i^2 \approx e^a \bar{X}_i^b;$$

therefore:

$$\text{Var}(\bar{D}) = \sum (P_i^2 (e^a \bar{X}_i^b / N_i)).$$

This yielded a variance and standard error for $\bar{D}$ that was more precise because the n stratum variances were estimated using just two parameters (the a and b of Taylor's Power Law), rather than the n σ_i^2 . This was particularly advantageous when some of the N_i were small (< 10) and the s_i^2 were very poor estimators of the σ_i^2 . The 95% confidence limits then became $\bar{D} \pm 1.96$ (SE). Because $\bar{D}$ was a weighted mean of independent means, we expected $\bar{D}$ to have a normal distribution (Central Limit Theorem).

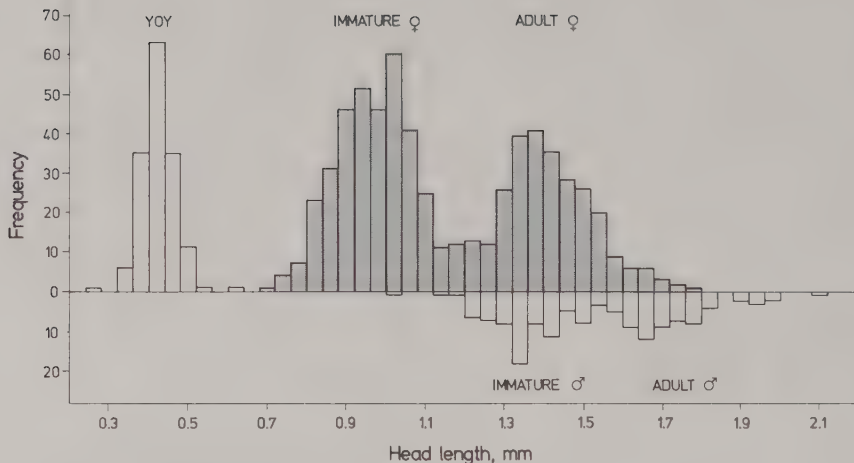


FIG. 5. Frequency distribution of head length (0.04-mm bin size) for *Gammarus* in P&N Lake, August 1979.

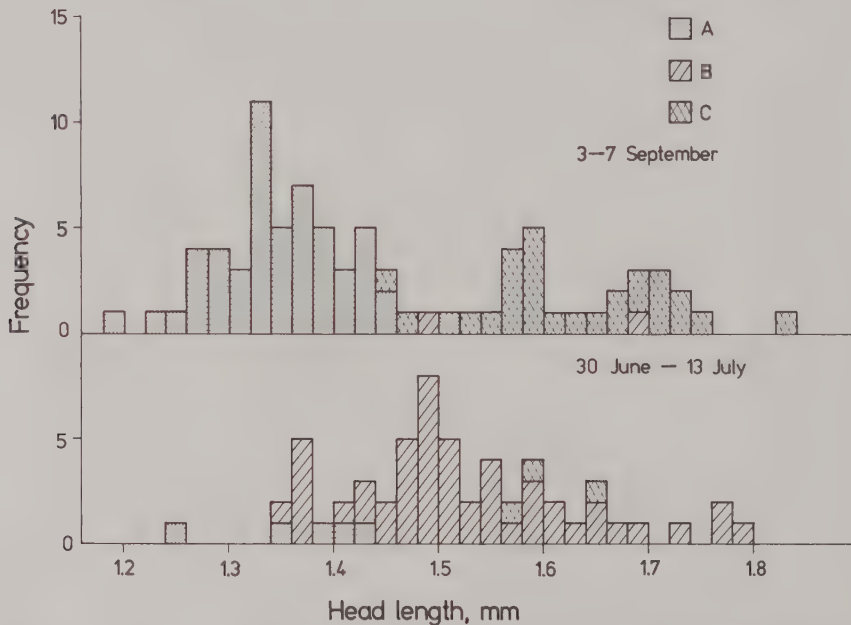


FIG. 6. Frequency distribution of head length (0.02-mm bin size) for female *Gammarus* in P&N Lake, 1977. A = immature, oostegites small and nonsetose; B = mature adults, oostegites large and setose, with or without young in brood pouch; C = postreproductive adults, oostegites large, setae lost.

Results

Substrate and Depth Effects

Sample variances were high, with standard deviations approximately equal to the mean population estimates at all depths. Both Trichoptera and *Gammarus* were most abundant on "cover" substrate compared with bare rock or flocculent sediment. The abundance of both groups was highest in shallow (2-4 m) water and decreased in the deepest part of each lake. This depth effect was partly dependent on substrate type because the preferred "cover" substrate was present only in shallow water. However, it was also partly independent of substrate type because shallow flocculent bottoms held more animals, especially *Gammarus*, than did deep flocculent bottoms.

Gammarus lacustris

No *Gammarus* were found in Jade Lake and only a few were found in Far Lake in the first year of sampling (1977), but they were abundant in P&N and Spring lakes.

The frequency distribution of head size showed a distinct peak for YOY and two overlapping peaks for immature and adult animals in any given year (e.g. Fig. 5), allowing us to distinguish cohorts. Early in July, nearly all adult females had large setose oostegites, either with or without young in the brood pouch (Fig. 6). By September the oostegites of adult females had shed their setae, and immature females with small oostegites constituted an identifiable smaller size class (Fig. 6). Mature females in August were never found with eggs in the

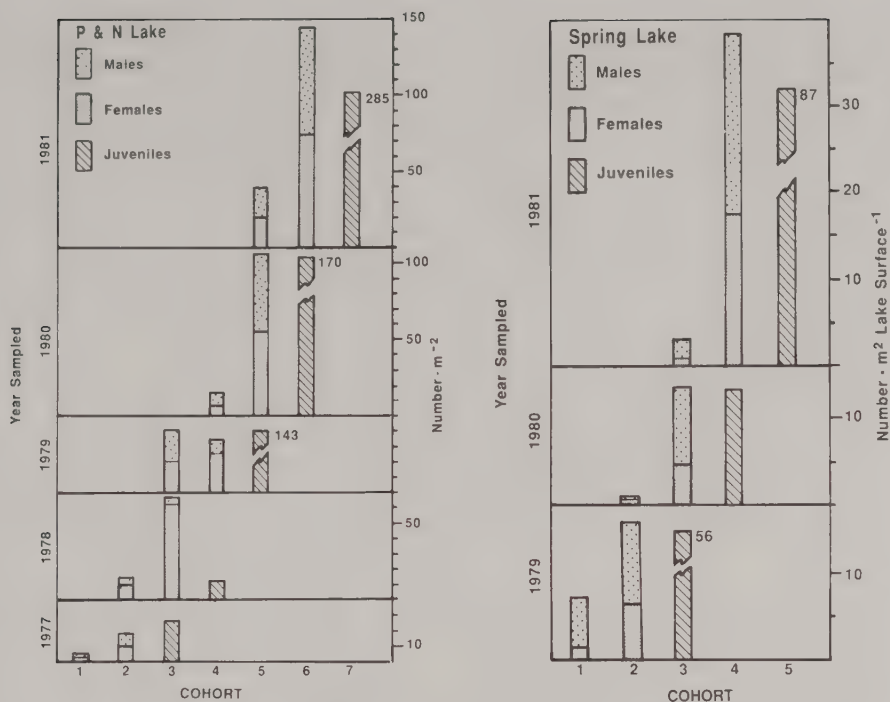


FIG. 7. Cohort (year class) strength of *Gammarus* in P&N and Spring lakes. Sampling dates were the same as in Fig. 9.

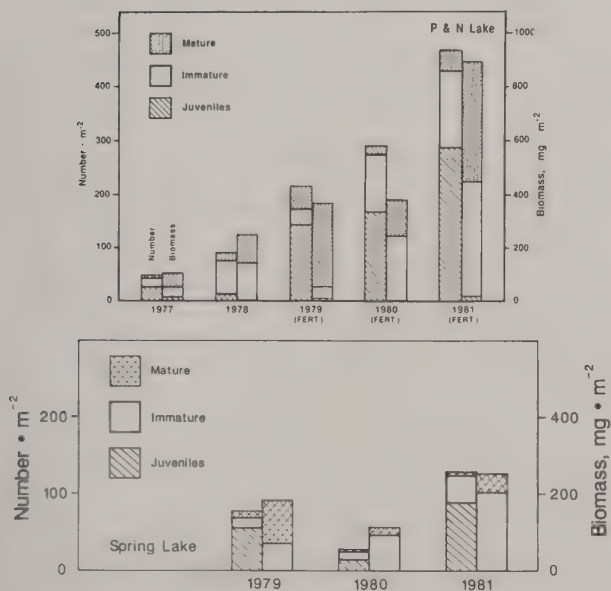


FIG. 8. Biomass and abundance of *Gammarus* in P&N and Spring lakes, 1977-81.

brood pouch, but only with young that were near the point of release. We also noted that samples taken in late August contained relatively few adults compared with samples taken in early August, indicating that adults died soon after reproducing. *Gammarus* in Spring and P&N lakes therefore had a 2-yr life cycle; young were released in mid to late summer, were identifiable as immature males or females the following sum-

mer, and reached sexual maturity the second year (Fig. 6), after which they died.

Cohort analysis showed that there were always more immatures than adults in a cohort, but in three instances (cohorts 3 and 4 in P&N Lake; cohort 4 in Spring Lake), we found fewer YOY than immatures in the same cohort the following year (Fig. 7). This may have resulted from undersampling of YOY by gear selectivity, random variation, or errors in interpretation.

Abundance of *Gammarus* was on the order of 50-100 individuals·m⁻² and 0.2 g dry wt·m⁻² in both lakes without fertilization (Fig. 8). During the first 3 yr of fertilization in P&N Lake, the abundance of YOY increased greatly, followed by increases in immatures and adults, resulting in about a fivefold increase in abundance and fourfold increase in biomass by the third year of fertilization (Fig. 8).

Trichoptera

Grensia comprised about 90% of the caddis numbers in all lakes, ranging from ~10·m⁻² in Jade Lake to ~100·m⁻² in Spring Lake and 93-95% of the biomass in all lakes (Fig. 9 and 10). Ninety percent of the remainder was *Apatania*, with *Hesperophylax* present only at very low densities, 0.1-1.0 individuals·m⁻². Caddis abundances from year to year were quite constant in all lakes except for P&N Lake towards the end of fertilization (Fig. 10). *Apatania* first instar began to increase in the second year of fertilization (1980) and continued increasing in 1981, followed by increasing numbers of fifth instar by 1981 (Fig. 9). *Grensia* showed no changes in P&N Lake until 1981, when the abundance of first and third instars increased (Fig. 9). The presence of fourth- and fifth- together with first-instar *Apatania* larvae in August indicates a life cycle of 2 yr or more. The large numbers of second- and third- in addition

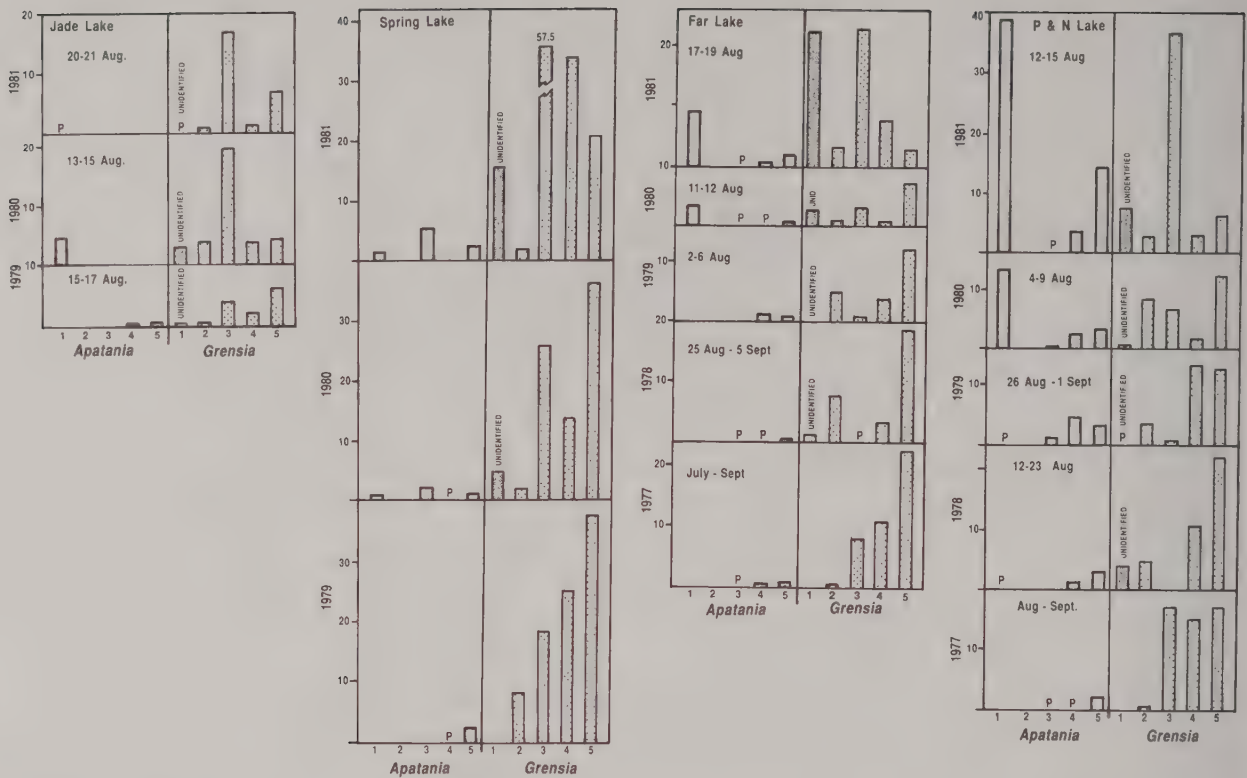


FIG. 9. Abundance per square metre of lake surface for the five instars of *Apatania zonella* and *Grensia praeterita* in Saqvaqujac experimental lakes. First-instar *Grensia* and second-instar *Apatania* were indistinguishable but were likely *Grensia* and are shown here as "unid." "P" stands for present, but in numbers too low to graph.

TABLE 2. *Gammarus lacustris* numbers, biomass, and calculated production and *P:B* according to the models of Morin and Bourassa (1992)^a and Plante and Downing (1989)^b.

Lake	Date	No·m ⁻²	Biomass (g dry wt·m ⁻²)	Production ^a	<i>P:B</i> ^a	Production ^b	<i>P:B</i> ^b
P&N	Aug. 1977	48.1	0.1118	0.2043	1.83	0.1950	1.74
	Aug. 1978	90.6	0.2500	0.4344	1.74	0.3683	1.47
	Aug. 1979	217.5	0.3670	0.7566	2.06	0.4988	1.36
	Aug. 1980	289.2	0.7493	1.3447	1.79	0.8766	1.17
	Aug. 1981	468.7	0.8900	1.7783	2.00	1.0043	1.13
				$\bar{X} =$	1.88	$\bar{X} =$	1.37
Spring	Aug. 1979	78.2	0.1882	0.3595	1.91	0.3153	1.68
	Aug. 1980	27.7	0.1132	0.1797	1.59	0.2110	1.86
	Aug. 1981	129.2	0.2573	0.5258	2.04	0.4037	1.57
				$\bar{X} =$	1.85	$\bar{X} =$	1.70

^a $\log P = -0.75 + 1.01 \log B - 0.34 \log M + 0.037 T$; B = biomass (g dry mass·m⁻²); M = mean individual mass (g); T = mean annual temperature (Table 1).

^b $\log P = 0.06 + 0.79 \log B - 0.16 \log (Wm) + 0.05 T$; B = biomass (g dry mass·m⁻²); Wm = adult mass (mg) (from Fig. 4); T = mean annual temperature.

to fourth- and fifth-instar *Grensia* larvae are consistent with a life cycle of at least 3 yr (Fig. 10).

No response by Trichoptera to decreased primary production after 2 yr of fertilization was detectable in Jade Lake (Fig. 10). This is not surprising, considering the 3-yr response time in P&N Lake.

Discussion

Calculation of Production

It is not possible to obtain precise estimates of production from our data because sampling only occurred annually, thereby precluding the detailed estimates of mortality required for the

TABLE 3. Trichoptera numbers, biomass, and calculated production and $P:B$ according to the models of Morin and Bourassa (1992)^a and Plante and Downing (1989)^b. Adult wt = 9 mg (Fig. 4).

Lake	Date	No·m ⁻²	Biomass (g dry wt·m ⁻²)	Production ^a	$P:B^a$	Production ^b	$P:B^b$
P&N	Aug. 1977	53.1	0.0782	0.1663	2.13	0.1596	2.04
	Aug. 1978	47.1	0.1087	0.1990	1.83	0.2070	1.90
	Aug. 1979	40.3	0.0578	0.1236	2.14	0.1257	2.17
	Aug. 1980	49.9	0.0732	0.1557	2.13	0.1515	2.07
	Aug. 1981	116.0	0.1948	0.3997	2.05	0.3282	1.68
					$\bar{X} = 2.06$		$\bar{X} = 1.97$
Spring	Aug. 1979	94.0	0.2689	0.4861	1.81	0.4536	1.69
	Aug. 1980	89.8	0.2588	0.4664	1.80	0.4401	1.70
	Aug. 1981	142.2	0.2146	0.4810	2.24	0.3796	1.77
					$\bar{X} = 1.95$		$\bar{X} = 1.72$
Far	Aug. 1977	43.6	0.1019	0.1905	1.87	0.2036	2.00
	Aug. 1978	33.5	0.0982	0.1699	1.73	0.1977	2.01
	Aug. 1979	25.0	0.0454	0.0917	2.02	0.1075	2.37
	Aug. 1980	19.7	0.0722	0.1154	1.60	0.1551	2.15
	Aug. 1981	71.0	0.0783	0.1884	2.41	0.1653	2.11
					$\bar{X} = 1.93$		$\bar{X} = 2.15$
Jade	Aug. 1979	14.2	0.0840	0.1153	1.37	0.1768	2.10
	Aug. 1980	42.3	0.0406	0.1026	2.53	0.0996	2.45
	Aug. 1981	27.4	0.0822	0.1420	1.73	0.1738	2.11
					$\bar{X} = 1.88$		$\bar{X} = 2.22$

^a $\log P = -0.75 + 1.01 \log B - 0.34 \log M + 0.037 T$; B = biomass (g dry mass·m⁻²); M = mean individual mass (g); T = mean annual temperature (Table 1).

^b $\log P = 0.06 + 0.79 \log B - 0.16 \log (Wm) + 0.05 T$; B = biomass (g dry mass·m⁻²); Wm = adult mass (mg) (from Fig. 4); T = mean annual temperature.

construction of seasonal mass–abundance curves (Allen 1951). However, a minimum production of *Gammarus* in P&N and Spring lakes can be calculated by summing the growth of the maturing cohort, the biomass of the immature cohort, and the biomass of juveniles, assuming that all three components were produced during the previous 12 mo. This procedure gave production to biomass ratios ($P:B$) ranging from 0.9 to 1.26 and averaging 1.05 for six lake years.

This compares favorably with values predicted from the literature. Johnson and Brinkhurst (1971) determined the relationship between $P:B$ and annual mean temperature for lake benthos, to which can be added Johnson's (1988) data for the amphipod *Pontoporeia hoyi* in Lake Huron. That relationship predicts a $P:B$ of about 1.1 at 3.4°C and 1.2 at 4.0°C, the mean annual temperatures of Spring and P&N lakes (Table 1). Banse and Mosher (1980) summarized a much larger data set and found a strong predictive relationship between $P:B$ and body mass. Their fig. 1 predicts $P:B$ near 1 for Spring and P&N lakes.

We have also calculated annual production according to two recent models. The Plante and Downing (1989) model uses mean annual temperature, mean annual biomass, and adult weight whereas the Morin and Bourassa (1992) model uses mean annual temperature, mean annual biomass, and mean individual weight. In both cases the results give $P:B$ on the order of 1.5–2.0 (overall mean $P:B = 1.68$ for *Gammarus*, 2.0 for Trichoptera) (Tables 2 and 3).

We conclude that the actual production of *Gammarus* and Trichoptera in Saqvaqujac lakes was somewhere between one and two times the annual biomass, with a $P:B$ on the order of 1.5 being most likely.

Response of Macroinvertebrates to Fertilization

The prefertilization biomass of *Gammarus* in Saqvaqujac lakes was about 0.2 g·m⁻² (Fig. 8). By the third year of fertilization in P&N Lake, it had increased to 0.9 g·m⁻², so the biomass change was about twice as great as the twofold increase in phytoplankton production (Welch et al. 1989). Trichoptera biomass only increased twofold, but further increases were likely, with equilibrium not occurring for several more years (Fig. 10). Chironomid emergence also increased about fourfold in P&N Lake after fertilization (Welch et al. 1988). Therefore, it appears that the increase in benthos energy flow was proportionately larger than the increase in primary production in Saqvaqujac lakes, implying an increase in food chain efficiency. However, we have not yet accounted for fish, whose energy basis was almost exclusively macroinvertebrates, or smaller fish that ate macroinvertebrates. Fish populations increased in P&N Lake as a result of fertilization but, in keeping with their relatively long life cycles, had not stabilized at the time of our last sampling in 1983 (K. Martin, H. Welch, and K. Mills, unpubl.). Quite possibly, if P&N Lake had been allowed to reach equilibrium under the new production regime, both chironomid emergence and macroinvertebrates biomass might have

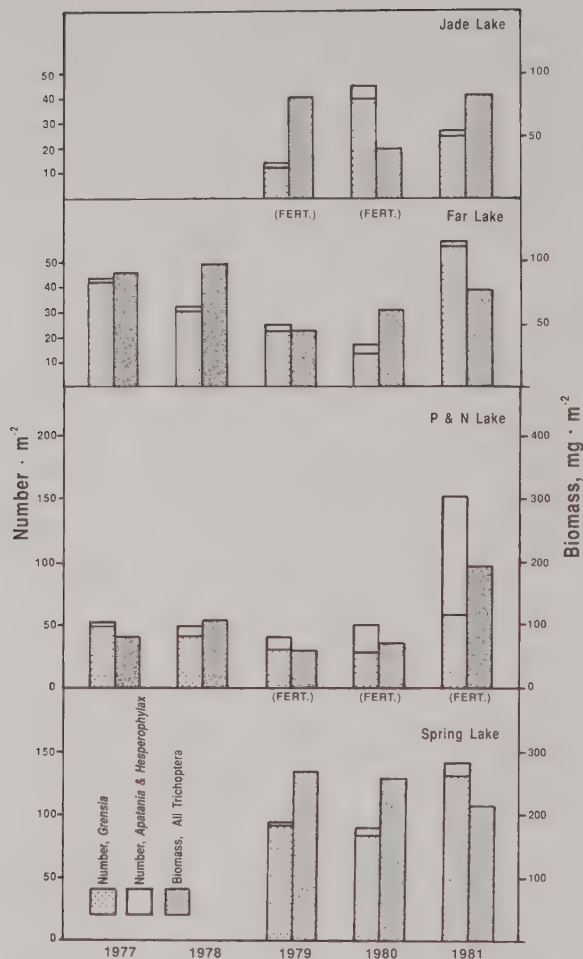


FIG. 10. Trichoptera abundance and dry weight biomass per square metre of lake surface, Saqvaquac lakes, 1977–81.

decreased somewhat from maximum values as fish predation adjusted upwards in response to increased prey availability.

We conclude that increased primary production resulting from the fertilization of arctic lakes increases the production of

macrobenthos in turn, with the equilibrium response time approximately twice the generation time of each species, ranging from 2 yr for Chironomidae (generally a 1-yr life cycle), 4 yr for *Gammarus* (2-yr life cycle), and even longer for Trichoptera (3-yr or more life cycle).

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References

- ALLEN, K. R. 1951. The Horokiwi Stream. A study of a trout population. N.Z. Mar. Dep. Fish. Bull. 10: 1–238.
- BANSE, K., AND S. MOSHER. 1980. Adult body mass and annual production/biomass relationships of field populations. Ecol. Monogr. 50: 355–379.
- JOHNSON, M. G. 1988. Production by the amphipod *Pontoporeia hoyi* in South Bay, Lake Huron. Can. J. Fish. Aquat. Sci. 45: 617–624.
- JOHNSON, M. G., AND R. O. BRINKHURST. 1971. Production of benthic macroinvertebrates of Bay of Quinte and Lake Ontario. J. Fish. Res. Board Can. 28: 1699–1714.
- MORIN, A., AND N. BOURASSA. 1992. Empirical models of annual production and *P:B* ratios for running water benthic invertebrates. Can. J. Fish. Aquat. Sci. 49: 532–539. (English translation from Can. Inst. Sci. Tech. Inf., National Research Council, Ottawa).
- NIMMO, A. P. 1986. Preliminary annotated checklist of the Trichoptera of the Northwest Territories, Canada. Muskox 43: 95–100.
- PLANTE, C., AND J. A. DOWNING. 1989. Production of freshwater invertebrate populations in lakes. Can. J. Fish. Aquat. Sci. 46: 1489–1498.
- SCHINDLER, D. W. 1980. Evolution of the Experimental Lakes Project. Can. J. Fish. Aquat. Sci. 37: 313–319.
- STEELE, R. G., AND J. H. TORRIE. 1980. Principles and procedures of statistics. McGraw-Hill, Toronto, Ont. 633 p.
- WELCH, H. E. 1985. Introduction of limnological research at Saqvaquac, northern Hudson Bay. Can. J. Fish. Aquat. Sci. 42: 506–520.
- WELCH, H. E., J. K. JORGENSEN, AND M. F. CURTIS. 1988. Emergence of Chironomidae (Diptera) in fertilized and natural lakes at Saqvaquac, N.W.T. Can. J. Fish. Aquat. Sci. 45: 731–737.
- WELCH, H. E., J. A. LEGAULT, AND M. A. BERGMANN. 1987. Effects of snow and ice on the annual cycles of heat and light in Saqvaquac lakes. Can. J. Fish. Aquat. Sci. 44: 1452–1461.
- WELCH, H. E., J. A. LEGAULT, AND H. J. KLING. 1989. Phytoplankton, nutrients and primary production in fertilized and natural lakes at Saqvaquac, N.W.T. Can. J. Fish. Aquat. Sci. 46: 90–107.
- WIGGINS, G. B. 1977. Larvae of the North American caddisfly genera (Trichoptera). University of Toronto Press, Toronto, Ont. 401 p.

Predicting End-of-Summer Oxygen Profiles in Stratified Lakes

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Molot, L. A., P. J. Dillon, B. J. Clark, and B. P. Neary. 1992. Predicting end-of-summer oxygen profiles in stratified lakes. *Can. J. Fish. Aquat. Sci.* 49: 2363–2372.

Mean end-of-summer dissolved oxygen profiles in the hypolimnion of oligotrophic and oligomesotrophic thermally stratified lakes were accurately predicted with a multivariate regression model. The model integrates the effects of lake morphometry, total phosphorus concentration (TP), and initial O_2 concentrations at spring turnover to predict mean end-of-summer O_2 concentration within individual hypolimnetic strata. Lake morphometry exerts a large influence on O_2 profiles and this influence is particularly evident in shallow (<20 m maximum depth) oligotrophic lakes. Predictions of O_2 profiles are sensitive to changes in TP concentrations, with all study lakes predicted to have severely O_2 -depleted hypolimnions by the end of summer at an epilimnetic TP of only $15 \mu\text{g}\cdot\text{L}^{-1}$.

Les profils de la teneur en oxygène dissous moyenne à la fin de l'été dans l'hypolimnion des lacs oligotrophes et oligomésotrophes à stratification thermique ont été prédits avec précision par un modèle de régression à plusieurs variables. Le modèle intègre les effets de la morphométrie du lac, de la concentration en phosphore total (PT) et des concentrations initiales d' O_2 au moment du renversement printanier pour prédire la concentration moyenne d' O_2 à la fin de l'été dans chaque strate hypolimnique. La morphométrie du lac exerce une influence importante sur les profils de la teneur en O_2 , influence qui est particulièrement évidente dans les lacs oligotrophes peu profonds (profondeur <20 m). Les prédictions des profils de la teneur en O_2 sont sensibles aux changements de la concentration de PT, et on avait prédit que, dans tous les lacs étudiés, l'hypolimnion serait gravement appauvri en O_2 à la fin de l'été avec une teneur en PT épilimnique de $15 \mu\text{g}\cdot\text{L}^{-1}$ seulement.

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Loss of hypolimnetic dissolved oxygen is an important measure of environmental stress in lakes, both because of the relationship between O_2 concentration and biological stress and because O_2 levels control many chemical oxidation/reduction reactions in aquatic habitats. In stratified lakes, O_2 profiles and consumption rates in the water column and sediments of the hypolimnion are functions primarily of labile organic input, which in turn is a function of nutrient concentration, lake morphometry, and allochthonous organic input.

Managing human activity in the catchments of thermally stratified oligotrophic and oligomesotrophic lakes requires, in part, understanding relationships between land use and dissolved O_2 depletion and subsequently developing and utilizing tools capable of predicting O_2 profiles. In the past, researchers have relied on measures of O_2 depletion such as the areal hypolimnetic oxygen depletion rate (AHOD) as a trophic status indicator (Hutchinson 1957; Welch and Perkins 1979). AHOD is defined as the hypolimnetic mass O_2 depletion rate divided by the hypolimnetic surface area. AHOD theoretically facilitates comparison of lakes of differing morphometry (Strom 1931; Hutchinson 1957). Other trophic status indicators, such as chlorophyll and total phosphorus (TP) concentrations, are, however, more frequently used today, and the AHOD has been little used as a management tool. Several studies have examined winter areal O_2 depletion rates (WODR) (e.g. Mathias and Barica 1980; Barica 1984; Babin and Prepas 1985).

Several regression models which predict AHOD or WODR as functions of morphometry or nutrient loading parameters have been reported (Cornett and Rigler 1979, 1980; Welch and

Perkins 1979; Charlton 1980; Mathias and Barica 1980; Barica 1984; Babin and Prepas 1985). However, the AHOD/WODR concept suffers from several deficiencies. First, spurious correlations can exist between AHOD or WODR and morphometric parameters such as mean hypolimnetic depth. For example, we created a simulated data set of 100 lakes with randomly generated O_2 consumption and lake morphometry. The correlation between AHOD and mean hypolimnetic depth in this data set was 0.99. A second deficiency in AHOD/WODR models is that they are designed to predict a hypolimnetic mass O_2 consumption rate rather than an O_2 profile. Charlton's (1980) AHOD model can, however, be modified to predict areal O_2 deficits for individual strata, and therefore, hypolimnetic O_2 profiles, by substituting strata volume/sediment surface area for hypolimnetic thickness (M. Charlton, pers. comm.).

This paper presents a multivariate regression model developed for a set of thermally stratified oligotrophic and oligomesotrophic lakes in central Ontario. The model predicts the end-of-summer O_2 profile based on lake morphometry, mean epilimnetic or spring turnover TP concentration, and mean O_2 concentrations at spring turnover. The model can also be used to predict AHOD.

Study Lakes

The study lakes are located in central Ontario in Haliburton County or the District of Muskoka (Fig. 1). The catchments are primarily forested with some cottage development and are underlain by Precambrian metamorphic silicate bedrock (Dillon

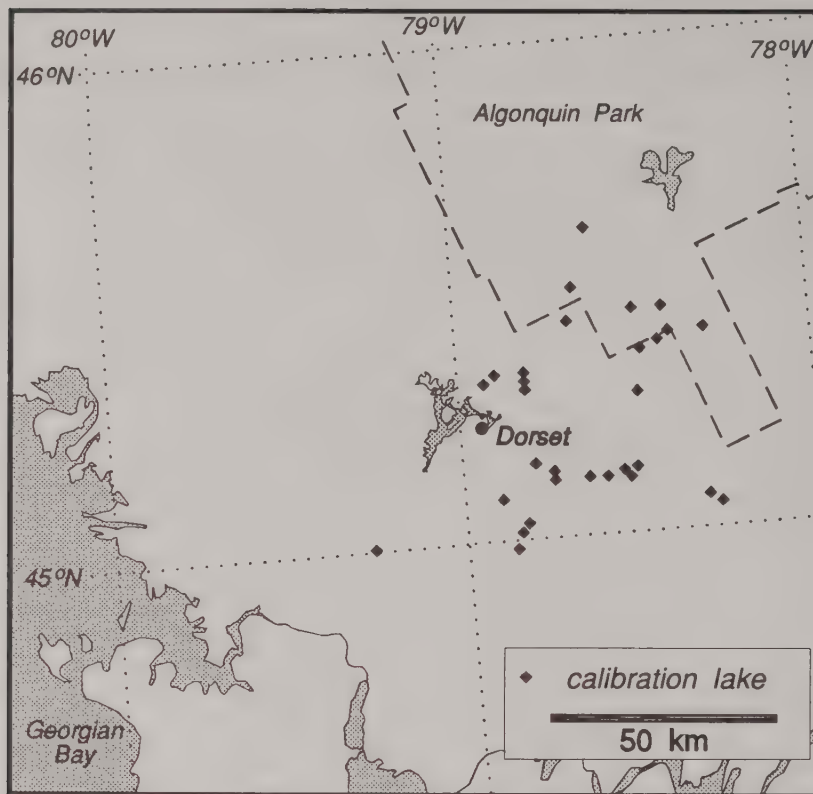


FIG. 1. Map of central Ontario showing location of the study lakes.

and Molot 1990), except for Glen Lake, which is partially underlain by marble.

The study lakes are divided into three groups: A, B, and C. The seven "A" lakes were monitored most intensively and the 17 "C" lakes the least intensively, while monitoring of the eight "B" lakes was of intermediate intensity. The "C" lakes include larger, deeper lakes, but they are exclusively oligotrophic.

The initial rationale for the division of the "A" and "B" lakes was that biogeochemical models (e.g. mass balance models) developed for the "A" lakes were to be tested on the "B" lakes. The range of trophic status, morphometry, and other biological and chemical conditions are similar in the "A" and "B" sets of lakes (Table 1).

The model was developed using 53 strata in one data set (seven "A" lakes, one with two basins) and tested with an independent data set of 53 strata (eight "B" lakes). "C" lakes (17 lakes, 83 strata) were used to test and further refine the spring O_2 model but not the end-of-summer O_2 model because oligotrophic lakes would then have been overrepresented.

The "A" and "B" lakes are softwater (mean conductivity ranging from 28 to 46 μS , except Glen which is 169 μS) and oligotrophic to slightly mesotrophic with long-term mean annual TP_{epi} ranging from 4.4 to 11.8 $\mu g P \cdot L^{-1}$ (Table 1), a long-term mean spring turnover TP (TP_{so}) ranging from 5.6 to 28.4 $\mu g P \cdot L^{-1}$, and ice-free chlorophyll *a* (Chl_{if}) ranging from 1.8 to 4.7 $\mu g \cdot L^{-1}$ (Molot and Dillon 1991). Long-term mean epilimnetic TN/TP (TN = total Kjeldahl N + NO_3) ranged from 73 to 175 (except 32 in Blue Chalk); hence, the lakes are considered P limited. Mean Secchi depth during summer strat-

ification ranged from 2.8 m in Dickie to 7.2 m in Solitaire and mean annual whole-lake dissolved organic carbon (DOC) ranged from 1.75 $mg C \cdot L^{-1}$ in Blue Chalk to 5.2 $mg C \cdot L^{-1}$ in Gullfeather (Table 1).

In the "C" lakes, TP_{epi} ranged from 3.3 to 5.8 $\mu g \cdot L^{-1}$, DOC from 1.5 to 5.2 $mg \cdot L^{-1}$, Secchi from 3.6 to 8.6 m, surface area from 18 to 697 ha, and maximum depth from 15 to 54 m (Table 1).

Methods

One station in the central basin of each "A" and "B" lake was sampled from 1976 to 1989 with the exception of Red Chalk, which was sampled in both the main and east basins, two distinct basins separated by a shallow (<2 m) narrow channel. Sampling frequency varied from weekly to monthly, depending on the year. Samples were collected at 2-m intervals and analyzed for O_2 concentration by the Winkler titration method (APHA 1971). Chl_{if} was measured at least monthly in volume-weighted samples representing twice the Secchi depth and averaged within each lake for the ice-free period, which included both spring and fall overturn. TP_{epi} was measured in volume-weighted epilimnetic samples. The epilimnion depth was determined from temperature profiles. Whole-lake TP was measured once during most years at spring turnover (TP_{so}). Sampling procedures are described in Dillon et al. (1988) and all analytical methods are described in Ontario Ministry of the Environment (1983).

One station in the central basin of each "C" lake was sampled periodically between 1978 and 1991 for TP, DOC, O_2 ,

TABLE 1. Mean ($\bar{z}$) and maximum ($z_{\max}$) depths, lake surface area (A_0), mean epilimnetic TP (TP_{epi}), mean spring turnover TP (TP_{so}), mean Secchi depth during summer stratification, maximum distance across the lake on a line through the sampling station (MD), and mean annual DOC in the "A", "B", and "C" lakes (sampling of Plastic began in 1979, others in 1976).

	$\bar{z}$ (m)	$z_{\max}$ (m)	A_0 (ha)	TP_{epi} ($\mu\text{g}\cdot\text{L}^{-1}$)	TP_{so} ($\mu\text{g}\cdot\text{L}^{-1}$)	Secchi (m)	DOC ($\text{mg}\cdot\text{L}^{-1}$)	MD (km)
"A" lakes								
Blue Chalk	8.5	23	52.4	5.0	5.8	6.8	1.75	0.9
Chub	8.9	27	34.4	8.4	11.1	3.3	4.78	1.2
Crosson	9.2	25	56.7	9.1	12.7	3.6	4.12	1.0
Dickie	5.0	12	93.6	10.7	12.6	2.8	5.02	1.3
Harp	13.3	37.5	71.4	6.7	8.1	3.8	3.88	1.7
Plastic	7.9	16.3	32.1	4.8	6.2	6.8	2.27	1.2
Red Chalk Main	16.7	38	44.1	4.4	5.6	6.3	2.49	0.8
Red Chalk East	5.7	19	13.1	4.8	7.3	5.0	2.85	0.5
"B" lakes								
Basshaunt	7.7	24	47.3	6.5	7.5	4.4	4.18	0.7
Bigwind	10.7	32	111	6.8	8.0	4.7	2.85	2.6
Buck	10.9	30	40.3	5.7	7.2	5.9	3.17	1.2
Glen	7.2	15	16.3	8.1	28.4	4.7	3.76	0.6
Gullfeather	4.8	13	65.9	11.8	11.8	2.5	5.16	1.1
Little Clear	8.1	25	10.9	6.3	8.5	5.4	2.69	0.4
Solitaire	13.3	31	124	5.0	5.6	7.2	2.13	1.5
Walker	6.2	17	68.2	5.8	6.7	5.3	3.38	1.1
"C" lakes								
Big Porcupine	7.5	30.5	235	3.4		6.4	2.1	1.9
Bonnechere	6.4	21.4	105	4.1		5.3	3.2	2.0
Clear	12.4	33.0	88.4	4.3		8.6	1.6	1.2
Cradle	12.4	33.0	17.9	5.2		8.2	1.5	0.9
Crown	8.0	30.0	136	3.7		6.9	1.9	1.1
Crystal	4.3	17.1	41	4.5		6.0	3.1	1.3
Delano	7.1	18.6	23.9	5.6		3.7	4.4	0.8
Kimball	22.0	61.0	213	3.3		7.1	2.5	1.1
Leonard	6.9	15.2	195	5.8		5.1	3.2	1.7
Louisa	16.1	61	531	3.4		6.5	2.9	2.8
Maggie	10.2	31.0	128.6	3.8		7.0	1.5	1.8
Nunikani	7.9	24.0	116	3.8		6.3	2.5	2.2
Pincher	6.1	15.5	42.1	4.8		5.2	2.3	1.2
Red Pine	10.1	38.7	365	4.6		6.8	2.4	3.5
Sherborne	9.6	35.1	252	3.8		6.5	2.4	1.3
Smoke	16.2	55.0	697	5.1		5.0	3.0	4.4
Timberwolf	7.4	20.4	167	5.7		3.6	4.3	2.0

and Secchi. Spring and late summer O_2 profiles were available for at least three years in each of the "C" lakes.

Volumes of strata defined at 2-m intervals were calculated from the formula of Hutchinson (1957). Sediment surface area for each stratum was assumed to be the difference between the top and bottom surface areas. Only strata exhibiting O_2 depletion, defined as a decrease of at least $1 \text{ mg}\cdot\text{L}^{-1}$ between spring turnover (O_2 (so)) and within 1 wk of August 31 (O_2 (f)) were used in the regression analyses. This permitted the elimination of metalimnetic and hypolimnetic strata exhibiting significant photosynthetic O_2 production. August 31 was chosen as the end of summer because sampling is typically more frequent before August 31 than after and because cooling and destratification generally begins about this time. "End-of-summer" was as late as early October in the "C" lakes, although most profiles were taken before September 24. Spring overturn typically occurred between the last week of April and mid-May. The depth of the uppermost plane of the zone depleted of O_2 is termed z_{ox} . AHOD was calculated as the decrease in mass (grams per square metre per summer) of O_2 below z_{ox} between spring turnover and the end of summer.

Regression techniques were used to develop a model which would predict the end-of-summer O_2 concentration in strata below z_{ox} . The model could then be applied to each stratum individually in order to generate a profile. The objective was to develop a model with no spurious correlations and a high goodness of fit with few independent variables. We avoided using a long list of independent variables to mathematically force an increase in fit.

Type I linear regression analyses were performed with PC SAS using long-term annual means (1976–87 for Chl_{f} , summer stratified Secchi, and TP_{epi} or TP_{so} and 1977–89 for O_2). Molot and Dillon (1991) showed that regression goodness of fit (the coefficient of determination, R^2 , defined as sum of squares due to regression/total sum of squares corrected for the mean) for other trophic status relationships was much higher when long-term averages were used. Hence, these regression models were best viewed as steady-state models rather than models capable of predicting temporal (annual) trends or variability.

Stepwise regression analyses were conducted with an expanded list of mathematically untransformed independent variables and their reciprocals and squares. The expanded list

TABLE 2. Mean observed O_2 concentrations (1977–89) at spring overturn ($O_2(i)$) and the end of summer ($O_2(f)$), volume/sediment surface area ratios (VSA), % lake volume (mean and range) with $O_2(f) < 4$ mg $O_2 \cdot L^{-1}$ (mean and range), and average AHOD between spring turnover and August 31 for "A" lake strata.

Lake	Depth (m)	$O_2(i)$ (mg·L ⁻¹)	$O_2(f)$ (mg·L ⁻¹)	VSA (m)	Volume (%)	AHOD (g·m ⁻²)
Blue Chalk	15	8.6	5.6	9.42	5.8	16.27
	17	8.4	3.1	2.87	2.4–10.9	
	19	8.1	0.9	3.78		
Chub	5	9.0	6.9	9.29	10.1	26.78
	7	8.6	5.2	10.52	6.4–63.1	
	9	8.5	5.5	11.86		
	11	8.3	5.4	11.91		
	13	8.1	5.0	7.23		
	15	7.9	3.9	7.89		
	17	7.3	2.8	7.69		
	19	6.5	1.5	5.75		
	21	5.7	0.6	6.32		
	23	4.7	0.3	3.22		
Crosson	5	9.5	7.5	9.61	7.5	25.74
	7	9.0	5.4	7.75	4.2–12.0	
	9	8.9	5.3	10.40		
	11	8.9	5.1	9.13		
	13	8.9	4.9	7.81		
	15	8.7	4.5	6.16		
	17	8.5	3.2	7.09		
	19	8.5	1.8	5.41		
Dickie	5	9.5	5.5	3.80	17.3	17.09
	7	9.1	1.0	3.44	5.9–35.4	
	9	8.5	0.2	1.86		
Harp	9	9.1	6.9	12.48	6.6	33.77
	11	9.0	6.0	12.40	0–14.1	
	13	8.9	6.0	13.03		
	15	8.9	5.9	13.16		
	17	8.7	5.5	13.40		
	19	8.6	5.3	13.08		
	21	8.4	5.0	13.17		
	23	8.4	4.6	10.07		
	25	8.3	4.0	7.99		
	27	8.0	3.6	7.53		
Plastic	29	8.0	3.2	7.17		
	31	6.7	2.5	5.95		
	11	8.4	6.6	4.77	5.4	11.59
	13	8.1	1.2	2.69	1.2–12.8	
Red Chalk Main	15	7.8	0.1	0.90		
	13	8.3	7.3	24.50	3.2	14.68
	15	8.4	6.6	23.49	1.8–17.1	
	17	8.3	6.3	17.12		
	19	8.1	6.1	15.76		
	21	8.1	5.7	14.48		
	23	8.1	5.2	13.41		
	25	7.8	4.8	5.14		
	27	7.7	4.4	4.14		
	29	7.4	3.7	6.71		
Red Chalk East	31	5.9	1.9	5.79		
	9	6.2	1.4	4.07	19.3	13.19
	11	5.2	0.4	3.08	10.4–32.2	
	13	4.1	0.3	3.70		
	15	3.0	0.2	5.20		

of independent variables permitted a search for significant non-linear relationships. Repetitious independent variables were then removed; for example, if Secchi depth was selected first, then Secchi² and 1/Secchi were removed. Only one of the TP_{epi}

and Chl_{if} variables was used in the final models because both are considered to be indicators of primary productivity. Hence, if TP_{epi} were selected first, all Chl_{if}, TP_{epi}², and 1/TP_{epi} variables were discarded. A mathematical forcing of goodness of fit

TABLE 3. Mean observed O_2 concentrations (1977–89) at spring overturn ($O_2(i)$) and the end of summer ($O_2(f)$), volume/sediment surface area ratios (VSA), % lake volume (mean and range) with $O_2(f) < 4$ mg $O_2 \cdot L^{-1}$ (mean and range), and average AHOD between spring turnover and August 31 for "B" lake strata.

Lake	Depth (m)	$O_2(i)$ (mg·L ⁻¹)	$O_2(f)$ (mg·L ⁻¹)	VSA (m)	Volume (%)	AHOD (g·m ⁻²)
Basshaunt	5	9.9	8.5	6.88	5.0	17.03
	7	8.8	6.3	8.17	2.8–41.7	
	9	8.4	5.4	8.82		
	11	8.0	5.1	7.74		
	13	7.6	4.5	5.74		
	15	7.6	4.2	3.71		
	17	7.5	3.5	7.45		
	19	7.2	2.0	4.92		
	21	6.5	0.8	2.36		
Bigwind	9	9.9	6.9	10.30	0.2	27.50
	11	9.6	6.2	8.37	0–1.1	
	13	9.5	6.4	12.41		
	15	9.3	6.3	10.75		
	17	9.2	6.8	12.70		
	19	9.3	6.2	10.81		
	21	9.0	6.1	11.72		
	23	8.9	5.9	10.00		
	25	8.9	5.7	7.86		
	27	8.6	5.3	6.07		
	29	8.4	4.5	3.12		
	31	8.5	3.8	0.67		
Buck	11	8.3	6.3	13.44	12.7	25.42
	13	8.2	5.2	13.61	8.3–25.6	
	15	7.8	4.5	7.50		
	17	7.6	3.5	6.13		
	19	7.1	2.5	7.24		
	21	6.9	1.5	4.33		
	23	6.4	0.6	3.87		
Glen	25	6.1	0.5	5.51	17.6	0
	9	1.1	1.7	3.18		
	11	0.2	0.2	3.58		
	13	0.1	0.1	2.35		
Gullfeather	5	8.7	3.1	3.31	27.0	18.13
	7	7.9	0.4	4.33	17.4–35.3	
	9	6.8	0.2	2.10		
Little Clear	9	5.9	4.7	5.39	21.6	6.65
	11	3.8	1.1	4.90	18.5–28.7	
	13	1.8	0.3	4.20		
	15	0.9	0.2	4.20		
	17	0.4	0.2	5.47		
	19	0.2	0.2	4.30		
Solitaire	21	0.01	0.3	2.67	3.2	22.72
	15	10.0	9.0	18.91		
	17	9.8	8.1	17.49		
	19	9.7	7.5	16.12		
	21	9.6	6.9	15.06		
	23	9.2	5.6	12.24		
	25	9.2	4.3	8.39		
Walker	27	8.6	3.3	3.72	6.0	9.94
	9	8.9	4.4	3.62		
	11	8.0	2.2	2.95		
	13	7.2	0.7	2.02		
	15	6.8	0.1	0.88	0.4–25.9	

was avoided by removing repetitious variables.

Mean end-of-summer O_2 concentration ($O_2(f)$) and its logarithm for each stratum were regressed against $O_2(i)$, individual strata volume/sediment surface area ratios (VSA), distance

below z_{ox} , TP_{epi} or TP_{so} , Chl_{if} , and the epilimnetic TP mass expressed per lake surface area (TP_{sa}), and their squares and reciprocals. TP_{sa} may be an index of the amount of respiration of settled organic matter within the sediment of each stratum.

Mean spring turnover O_2 and its logarithm were regressed against lake surface area, maximum distance (MD, maximum distance across the lake in any direction on a line through the sampling station), depth of stratum below the lake surface, VSA , Chl_{if} , and TP_{epi} or TP_{so} ; z_{ox} and its logarithm were regressed against DOC, lake surface area, TP_{epi} , Chl_{if} , mean depth and maximum depth, and their squares and reciprocals. The dependent variables were logarithmically transformed to determine whether this mathematical transformation improved goodness of fit.

Results

The uppermost depth exhibiting some O_2 depletion (z_{ox}) ranged from 4 m in Chub, Crosson, Dickie, Basshaunt, and Gullfeather to 14 m in Blue Chalk and Solitaire (Tables 2 and 3). By the end-of-summer, mean O_2 was frequently less than $4 \text{ mg}\cdot\text{L}^{-1}$ at some depths, even in oligotrophic lakes. Water quality guidelines in use in Canada (Anonymous 1987) consider $4 \text{ mg}\cdot\text{L}^{-1}$ to be the concentration at which severe impairment of salmonid fish production occurs (stages other than embryo and larval) and the concentration at which moderate impairment of nonsalmonid fish production occurs (stages other than embryo or larval). The mean fraction of the total lake volume with O_2 concentrations $<4 \text{ mg}\cdot\text{L}^{-1}$ by the end-of-summer in the "A" and "B" lakes ranged from 0.2% in Bigwind to 27.0% in Gullfeather (Tables 2 and 3).

The size of the zone with $O_2(f) <4 \text{ mg}\cdot\text{L}^{-1}$ within each lake varied annually. The greatest annual variability was observed in Chub where the zone ranged from 6.4 to 63.1% of the total lake volume (Fig. 2a). In Harp, minimum and maximum O_2 depletion occurred in consecutive years (Fig. 2b). We have, in this paper, built and tested models using only long-term average O_2 data.

Mean AHOD in the "A" and "B" lakes (excluding Glen) was weakly correlated with trophic status variables such as Secchi (-0.33), TP_{epi} ($+0.24$), and Chl_{if} (-0.08) but was more strongly correlated with surface area ($+0.45$). The correlation

between AHOD and the percent of lake volume with $O_2(f) <4 \text{ mg}\cdot\text{L}^{-1}$ was -0.22 . Hence, in these lakes, AHOD does not appear free of the effects of lake morphometry and is also a poor trophic status indicator.

Spring turnover frequently resulted in undersaturated strata in some of the "A" and "B" lakes (Tables 2 and 3), indicating that turnover was incomplete. The extent to which reoxygenation occurred after ice-out was apparently a function of lake surface area and maximum distance. Spring turnover was very weak in Red Chalk East, Glen, and Little Clear (surface areas 11–16 ha, maximum distance $<0.6 \text{ km}$), partial mixing occurred in Chub, Red Chalk Main, Buck, Gullfeather, and Walker (surface areas 34–68 ha, maximum distance 0.8–1.2 km), and complete mixing occurred in the remaining seven "A" and "B" lakes (surface areas 52–124 ha, maximum distance $>0.9 \text{ km}$). Spring mixing was always very poor when the maximum distance was $\leq 0.6 \text{ km}$ and complete when the maximum distance was $\geq 1.3 \text{ km}$. Lakes in the intermediate range exhibited either partial or complete mixing. Similarly, spring mixing in the "C" lakes was always very poor when the maximum distance was $\leq 0.9 \text{ km}$ and complete when the maximum distance was $\geq 1.4 \text{ km}$.

Predicting z_{ox}

The regression for predicting z_{ox} the uppermost plane of the O_2 -depleted zone for the "A" lakes, was

$$(1) \quad z_{ox} = 1.52 + 28.1/\text{DOC} \\ R^2 = 0.91, \text{MSE} = 1.60, F = 59.6, P < 0.0002.$$

Equation 1 is consistent with DOC control of water clarity in nonturbid oligotrophic and mesotrophic waters (Brezonik 1978; Canfield and Hodgson 1983; Lind 1986). Predicted and observed z_{ox} in the "B" lakes were highly correlated using equation 1 ($r = 0.90$).

Spring O_2 Model

Because it was apparent that many of the study lakes were not completely reoxygenated each spring, we first attempted to

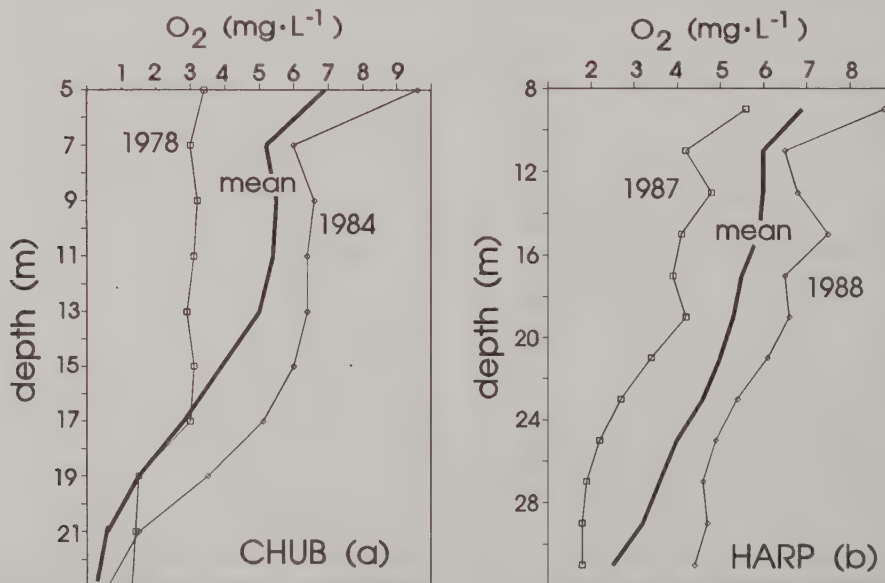


FIG. 2. Mean and range of observed end-of-summer O_2 profiles for (a) Chub Lake (1978 and 1984) and (b) Harp Lake (1987 and 1988).

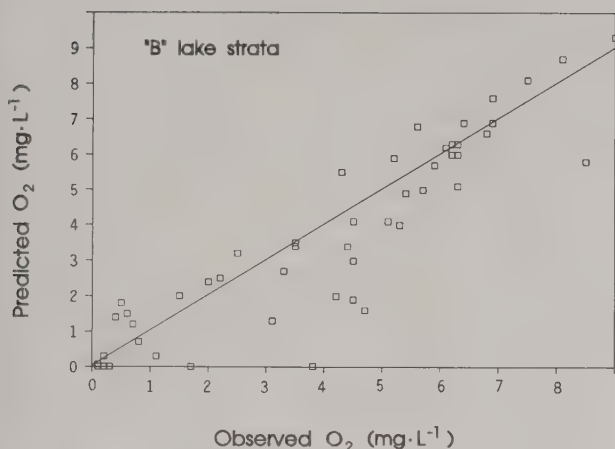


FIG. 3. Predicted versus mean observed end-of-summer O_2 concentration for individual "B" lake strata (equation 5) using mean observed O_2 concentration at spring turnover. The 1:1 line is shown.

develop a model to predict the initial O_2 concentrations at the start of each summer period, i.e. $O_2(i)$. We used data from the "A" lake set to develop the regression model

$$(2) \quad O_2(i)_z = 11.2 - 70.9/A_0 - 0.092z$$

$$R^2 = 0.80, \text{MSE} = 0.37, F = 102.0, P < 0.0001$$

where z is the depth below the lake surface. Equation 2 predicts that the O_2 concentration at the surface of an infinitely large lake at spring turnover is $11.2 \text{ mg} \cdot \text{L}^{-1}$ which is the approximate concentration of air-saturated water at 10°C .

We then tested the model developed using the "A" lake data set on the "B" lake data set. Predicted $O_2(i)$ was highly correlated with the long-term average $O_2(i)$ in the "B" lakes ($r = 0.88$); however, the poorest predictions were made for the bottom layers of the deepest lakes. The model predicted lower O_2 concentrations at spring turnover in these deep layers than were observed. We then tested the model using the "C" lake data set, since this set contained a number of deep lakes, including several deeper than those used for model development ("A" lakes). Again, poor agreement between measured and predicted concentrations was found in the deep layers. For example, observed and predicted O_2 concentrations at 43 m in Louisa (a "C" lake) were 10.3 and $7.1 \text{ mg} \cdot \text{L}^{-1}$, respectively. Similarly, observed and predicted O_2 concentrations at 43 m in Smoke (a "C" lake) were 11.1 and $7.1 \text{ mg} \cdot \text{L}^{-1}$, respectively. The correlation between observed and predicted spring turnover O_2 using the "A" lake model and "C" lake data was 0.63 .

As a consequence of the failure of the model in deep lakes, we combined "A" and "C" lake data for model development. Lakes with maximum distance (MD) $< 1.4 \text{ km}$ were separated from lakes with larger maximum distances; maximum distance was a better criterion for separation than lake surface area although surface area explained more variance in the regression. The choice of 1.4 km as the cut off above which maximum distance affects the initial O_2 concentration was made empirically by trial and error:

MD $< 1.4 \text{ km}$:

$$(3) \quad \log_{10}O_2(i)_z = 0.99 - 5.74/A_0 + 0.64/z$$

$$R^2 = 0.77, \text{MSE} = 0.0033, F = 116, P < 0.0001$$

MD $\geq 1.4 \text{ km}$:

$$(4) \quad \log_{10}O_2(i)_z = 1.07 - 6.95/A_0 - 0.0043z/\text{MD}$$

$$R^2 = 0.71, \text{MSE} = 0.00062, F = 74.2, P < 0.0001.$$

Using a decision tree approach in which either equation 3 or 4 was used depending on lake size, the correlation between observed and predicted $O_2(i)$ in the "B" lakes was 0.91 , and the predictions for the deep layers were as good as those for shallower layers.

End-of-Summer O_2 Model

We used the "A" lake data set to develop a model for the end-of-summer O_2 concentration. VSA, observed mean $O_2(i)$, and TP_{epi} were significant dependent variables in the $O_2(f)$ regression analysis. Goodness of fit for $O_2(f)$ was higher when $O_2(f)$ was logarithmically transformed. The regression model for predicting $O_2(f)$ concentration at depth z is

$$(5) \quad \log_{10}O_2(f)_z = 1.89 - 1.88/\text{VSA}_z - 7.29/O_2(i)_z - 0.0027\text{TP}_{\text{epi}}^2$$

$$R^2 = 0.88, \text{MSE} = 0.027, F = 124.9, P < 0.0001.$$

The order of presentation of the independent variables from left to right is the order in which they were selected by the stepwise procedure. The first two variables accounted for 86% of the variance. The stepwise procedure selected additional independent variables, but these were considered redundant and removed for the reasons outlined in the Methods section. Distance below z_{ox} was a significant independent variable, but accounted for less than 1% of the variance. When a stepwise procedure was applied to $\log_{10}O_2(f)$ with a limited independent variable list consisting of $1/\text{VSA}$, $1/O_2(i)$, TP_{epi}^2 , and distance below z_{ox} , the latter two variables were not selected by the procedure.

Because measurements of TP at spring overturn are sometimes more readily available, TP_{so} was substituted for TP_{epi} in regressions for $\log_{10}O_2(f)$ using "A" lake data. The regression is

$$(6) \quad \log_{10}O_2(f)_z = 1.83 - 1.91/\text{VSA}_z - 7.06/O_2(i)_z - 0.0013\text{TP}_{\text{so}}^2$$

$$R^2 = 0.88, \text{MSE} = 0.029, F = 116.9, P < 0.0001.$$

The regression is similar to that obtained with TP_{epi} because of the large influence exerted by lake morphometry in the study lakes.

Mean end-of-summer O_2 concentration is primarily a function of the stratum volume/sediment surface area ratio (VSA) and initial O_2 concentration (as approximated by the mean concentration at spring turnover), while TP_{epi} or TP_{so} is of secondary importance in the "A" lakes. TP_{epi} (or TP_{so}) exerts more influence on O_2 at higher TP_{epi} concentrations.

End-of-Summer O_2 Model Validation

We tested these models by comparing the predictions made using equations 5 and 6 with data collected for the "B" lake set. Model predictions were strongly correlated with observations (Fig. 3). The correlation of predicted long-term average, end-of-summer O_2 calculated using observed spring turnover O_2 was 0.91 using either of the two equations. The correlation between observed and predicted $O_2(f)$ in the "B" lakes, using predicted $O_2(i)$ in place of measured $O_2(i)$ (equations 3, 4, 5, and 6), was 0.88 . Similarly, the correlation between predicted $O_2(f)$ using observed $O_2(i)$ and observed $O_2(f)$ in the "C" lakes was 0.88 .

O_2 ($mg \cdot L^{-1}$)

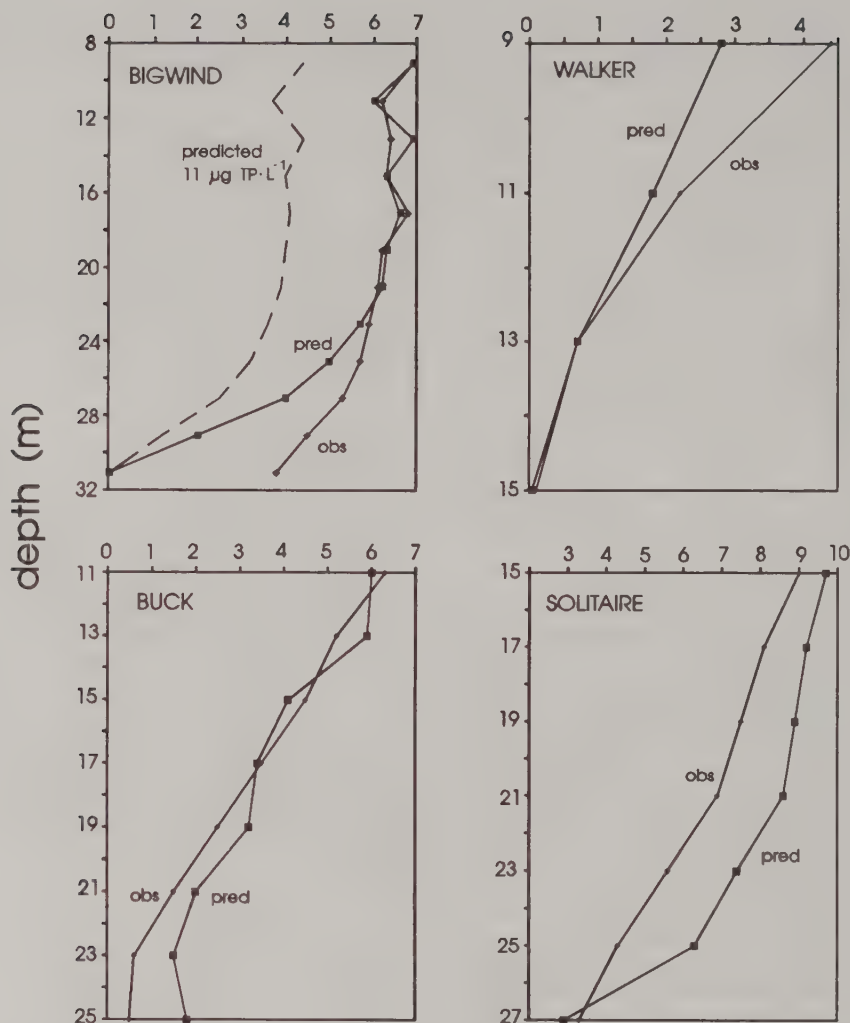


FIG. 4. Predicted and mean observed end-of-summer O_2 profiles for four "B" lakes using observed spring turnover O_2 (equation 5). The predicted Bigwind Lake O_2 profile is also shown for $11 \mu g TP \cdot L^{-1}$ (the observed average is $6.8 \mu g TP \cdot L^{-1}$).

Predictions for end-of-summer profiles for the "B" lakes were very similar to the long-term average measurements (Fig. 4). There were, however, a few cases where predictions deviate. Predicted end-of-summer O_2 for Bigwind at $6.8 \mu g TP \cdot L^{-1}$, the measured long-term average, was in good agreement with observed means (Fig. 4). Deviations from observations were small above 25 m, but predictions were at least $1 mg \cdot L^{-1}$ less than observations below 25 m which suggests (1) that distribution of new organic matter was not uniform over the lake bottom or (2) an input of oxygenated ground water to deep waters. However, this pattern was not consistent among lakes and was, in fact, reversed in Buck where predictions in deep strata were greater than observed O_2 concentrations.

The regression model was sensitive to increases in TP_{epi} . For example, in Bigwind (Fig. 4), the model predicted that with a

relatively small increase in TP_{epi} to $11 \mu g TP \cdot L^{-1}$, $O_2(f)$ would be less than $4 mg \cdot L^{-1}$ below 15 m. If TP_{epi} increased to $13 \mu g TP \cdot L^{-1}$, $O_2(f)$ would be less than $3.3 mg \cdot L^{-1}$ below z_{ox} . The model predicted $O_2(f) < 4 mg \cdot L^{-1}$ in all "B" lakes at a TP_{epi} of only $15 \mu g \cdot L^{-1}$.

Anoxia in the hypolimnia of deep lakes is caused by excessive nutrients and not by lake morphometry (assuming an insignificant labile organic carbon input) because deep, oligotrophic lakes have oxygenated hypolimnia. Therefore, assessing the accuracy of model responses to increases in TP would be best assessed by comparing predicted responses of large, deep, oligotrophic lakes with observations of large, deep, eutrophic lakes. Due to the lack of deep eutrophic lakes among the study sites, model accuracy was indirectly assessed by comparing the response of Bigwind, which is a deep oligotrophic lake, with

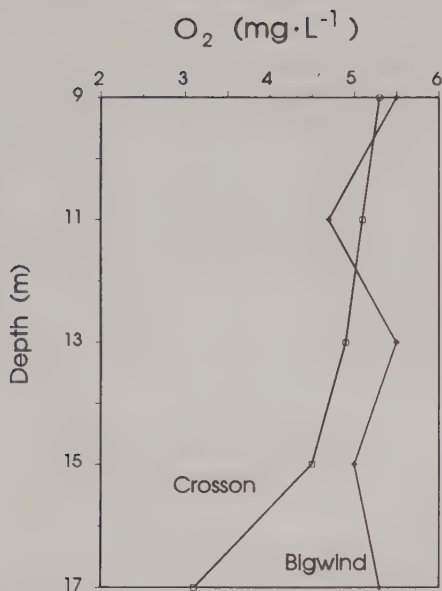


FIG. 5. Mean observed end-of-summer O_2 profile for Crosson Lake and the profile predicted for Bigwind Lake at $9.1 \mu\text{g TP}\cdot\text{L}^{-1}$ using observed spring turnover O_2 (equation 5).

observations of Crosson (Fig. 5), which is mesotrophic, although somewhat smaller (Tables 1, 2, and 3). In this scenario, the TP_{epi} concentration in Bigwind was set at $9.1 \mu\text{g TP}\cdot\text{L}^{-1}$, which was the average concentration in Crosson. The comparison of $O_2(f)$ was limited to strata between 9 and 17 m because bottom effects are evident in Crosson at 19 m (although the maximum depth in Crosson was 25 m, the sampling station was in shallower water; station depth was frequently <21 m). The profiles are similar between 9 and 15 m but diverge significantly at 17 m, presumably because of bottom effects at that depth in Crosson.

Discussion

This study clearly demonstrates that a relatively simple model consisting of three multivariate regression equations integrating the effects of initial O_2 concentration, lake morphometry, and TP concentration is capable of accurately predicting average end-of-summer O_2 profiles in small inland oligotrophic and oligomesotrophic lakes. It is not clear to what extent the model can be extrapolated beyond the range of conditions employed in this study (e.g. there are no mesotrophic lakes). Nevertheless, all of the lakes in this study were clearly influenced by morphometry, and this influence is particularly evident in shallow (<20 m maximum depth) oligotrophic lakes. The model also predicted severely O_2 -depleted hypolimnia in all "B" lakes by the end of summer at a TP_{epi} of only $15 \mu\text{g}\cdot\text{L}^{-1}$. If lakes with much higher TP_{epi} are included in subsequent model development, allochthonous organic loading should be tested as an independent variable because of the likelihood that organic loads are accompanied by a high biochemical O_2 demand.

The model suggests that the rate of supply of O_2 to the hypolimnion from upper waters is low after stratification, and therefore, respiration rates and consequently end-of-summer O_2 concentrations are strongly dependent on initial O_2 concentrations (Trimbee and Prepas 1988). Dependence of respiration rate on initial O_2 concentration is consistent with first-order

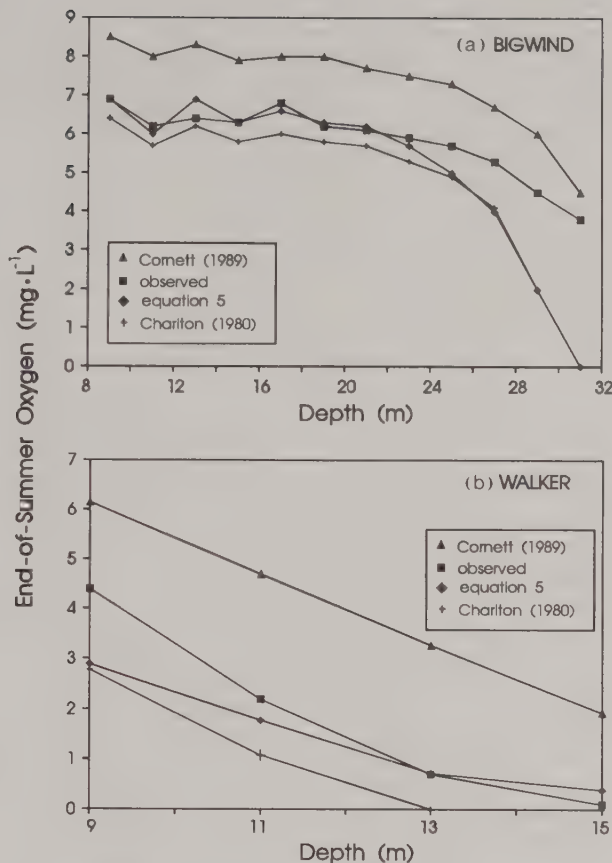


FIG. 6. Comparison of observed end-of-summer O_2 profiles with model predictions for (a) Bigwind Lake and (b) Walker Lake. Assumptions for the Cornett and Charlton models were 4°C , $3.2 \mu\text{g Chl}\cdot\text{L}^{-1}$, and $65 \text{ mg retained TP}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ for Bigwind and 6°C , $2.9 \mu\text{g Chl}\cdot\text{L}^{-1}$, and $57 \text{ mg retained TP}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ for Walker. The O_2 consumption period was set at 100 d.

enzyme kinetics (Engel 1977) and with diffusion control of O_2 into sediments (Hargrave 1969). Furthermore, horizontal transport rates in hypolimnetic waters are orders of magnitude larger than vertical transport rates (Imboden and Joller 1984). Hence, we assume that with the onset of thermal stratification, O_2 is supplied to the sites of respiration primarily from within the same horizontal plane as the respiration sites rather than from upper waters. As a result, small stratum volume/sediment surface area ratios are associated with large declines in O_2 concentration during the summer. This is consistent with significant O_2 depletion throughout the hypolimnion by the end of summer in shallow Walker (Table 3; Fig. 4), although mean TP_{epi} was only $5.8 \mu\text{g}\cdot\text{L}^{-1}$ (Table 1). The model also suggests that the spatial distribution of new organic substrate among sediments in the tropholytic zone (below z_{ox}) does not affect O_2 profiles in these lakes.

The relationship between AHOD and lake surface area may be explained by the dependence of spring overturn O_2 concentrations and, therefore, end-of-summer O_2 concentrations on lake surface area (equations 3, 4, and 5). It would be expected that two lakes of similar trophic status (i.e. similar TP_{epi} and chlorophyll), one with a very large surface area and one with a very small surface area, would have different AHODs because of the differences in spring overturn O_2 concentrations. There-

fore, neither AHOD nor O_2 profiles are free of the effects of lake morphometry. Cornett and Rigler (1980) and Charlton (1980) also concluded that AHOD is influenced by lake morphometry; therefore, while within-lake AHOD comparisons are valid, between-lake AHOD comparisons are less valuable.

Model predictions for "B" lakes were similar to predictions from Charlton's (1980) model but did not agree well with Cornett's (1989) O_2 model (Fig. 6). Cornett's model for predicting volumetric O_2 consumption rates for individual strata overestimated end-of-summer O_2 concentrations. Charlton's (1980) AHOD model, which was designed to predict lake AHOD, predicted areal O_2 deficits for individual strata and, therefore, hypolimnetic O_2 profiles reasonably well when individual strata VSA were substituted for hypolimnetic thickness. Hence, each stratum was considered to be a hypolimnion. Hypolimnetic thickness is equivalent to VSA when the entire hypolimnion is taken to be one stratum, but in our model, stratum thickness is fixed at 2 m and is independent of VSA. The three models are qualitatively similar because they predict O_2 consumption as a function of VSA and trophic status (chlorophyll and retained TP or epilimnetic TP concentration); however, our model does not include the effects of temperature.

Annual variation in hypolimnetic O_2 profiles complicates the task of predicting the effects of nutrient loading on fish production and survival because of the relatively large effect that extreme years might have on survival even though the long-term average O_2 profile may be acceptable. Years with severely impoverished O_2 conditions will likely occur more frequently as the long-term average TP increases; hence, strategies designed to maintain a minimum fisheries habitat volume by the end of summer should set average TP targets with annual fluctuations in mind or set very conservative values for minimum habitat. The model presented here could be used to derive upper limits to permissible TP increases.

The model does not provide information on annual variability. It is precisely because the model relies on long-term averages that it is so successful. The confounding effects of varying external factors such as temperature, wind and solar radiation, and varying internal factors which affect primary production, oxygen transport, and energy transfer to higher trophic levels are removed by the use of long-term averages.

The model is best regarded as a steady-state model which predicts long-term mean end-of-summer hypolimnetic O_2 profiles (and AHOD if desired) below z_{ox} in stratified oligotrophic and oligomesotrophic lakes within a geographic region. If a model is to be developed for other regions, a minimum of 6 yr of consecutive sampling for O_2 profiles and TP_{epi} or TP_{so} is recommended (Molot and Dillon 1991) to take into account the effects of extreme years such as those that occurred (consecutive years 1987 and 1988) in Harp (Fig. 2b; Table 3). The model coefficients we have presented are regional only, and application outside central Ontario entails deriving coefficients based on an appropriate local, long-term data set.

Acknowledgements

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References

- ANONYMOUS. 1987. Canadian water quality guidelines. Chap. 3. Freshwater aquatic life. Water Quality Objectives Division, Water Quality Branch, Environment Canada, Ottawa, Ont. K1A 0H3.
- APHA. 1971. Standard methods. 13th ed. American Public Health Association, New York, NY. p. 477-481.
- BABIN, J., AND E. E. PREPAS. 1985. Modelling winter oxygen depletion rates in ice covered temperate zone lakes in Canada. *Can. J. Fish. Aquat. Sci.* 42: 239-249.
- BARICA, J. 1984. Empirical models for prediction of algal blooms and collapses, winter oxygen depletion and a freeze-out effect in lakes: summary and verification. *Verh. Int. Ver. Limnol.* 22: 309-319.
- BREZONIK, P. L. 1978. Effect of organic carbon and turbidity on Secchi disc transparency. *J. Fish. Res. Board Can.* 35: 1410-1416.
- CANFIELD, D. E. JR., AND L. M. HODGSON. 1983. Prediction of Secchi disc depths in Florida lakes: impact of algal biomass and organic colour. *Hydrobiologia* 99: 51-60.
- CHARLTON, M. N. 1980. Hypolimnion oxygen consumption in lakes: discussion of productivity and morphometry effects. *Can. J. Fish. Aquat. Sci.* 37: 1531-1539.
- CORNETT, R. J. 1989. Predicting changes in hypolimnetic oxygen concentrations with phosphorus retention, temperature and morphometry. *Limnol. Oceanogr.* 34: 1359-1366.
- CORNETT, R. J., AND F. H. RIGLER. 1979. Hypolimnetic oxygen deficits: their prediction and interpretation. *Science (Wash., DC)* 205: 580-581.
- 1980. The areal hypolimnetic oxygen deficit: an empirical test of the model. *Limnol. Oceanogr.* 25: 672-679.
- DILLON, P. J., AND L. A. MOLOT. 1990. The role of ammonium and nitrate retention in the acidification of lakes and forested catchments. *Biogeochemistry* 11: 23-43.
- DILLON, P. J., K. H. NICHOLLS, B. A. LOCKE, E. DEGROSBOIS, AND N. D. YAN. 1988. Phosphorus-phytoplankton relationships in nutrient-poor soft-water lakes in Canada. *Verh. Int. Ver. Limnol.* 23: 258-264.
- ENGEL, P. C. 1977. Enzyme kinetics. Wiley, New York, NY. 46 p.
- HARGRAVE, B. T. 1969. Similarity of oxygen uptake by benthic communities. *Limnol. Oceanogr.* 14: 801-805.
- HUTCHINSON, G. E. 1957. A treatise on limnology. Vol. 1. John Wiley & Sons, Toronto, Ont. 1015 p.
- IMBODEN, D. M., AND TH. JOLLER. 1984. Turbulent mixing in the hypolimnion of Baldeggersee (Switzerland) traced by natural radon-222. *Limnol. Oceanogr.* 29: 831-844.
- LIND, O. T. 1986. The effect of non-algal turbidity on the relationship of Secchi depth to chlorophyll *a*. *Hydrobiologia* 140: 27-35.
- MATHIAS, J. A., AND J. BARICA. 1980. Factors controlling oxygen depletion in ice covered lakes. *Can. J. Fish. Aquat. Sci.* 37: 185-194.
- MOLOT, L. A., AND P. J. DILLON. 1991. Nitrogen/phosphorus ratios and the prediction of chlorophyll in phosphorus-limited lakes in central Ontario. *Can. J. Fish. Aquat. Sci.* 48: 140-145.
- ONTARIO MINISTRY OF THE ENVIRONMENT. 1983. Handbook of analytical methods for environmental samples. Vols. 1 and 2. Ontario Ministry of the Environment, Toronto, Ont.
- STROM, K. M. 1931. Feforvatn. A physiographic and biological study of a mountain lake. *Arch. Hydrobiol.* 22: 491-536.
- TRIMBEE, A. M., AND E. E. PREPAS. 1988. Dependence of lake oxygen depletion rates on maximum oxygen storage in a partially meromictic lake in Alberta. *Can. J. Fish. Aquat. Sci.* 45: 571-576.
- WELCH, E. B., AND M. A. PERKINS. 1979. Oxygen deficit - phosphorus loading relation in lakes. *J. Water Pollut. Control Fed.* 51: 2823-2828.

Bacteria as Biocontrol Agents for Rearing Larvae of the Crab *Portunus trituberculatus*

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Bacterial strain PM-4, isolated from a crustacean culturing pond, improved the growth of crab (*Portunus trituberculatus*) larvae and repressed the growth of *Vibrio anguillarum* in seawater. PM-4 was cultured in a large quantity and was added daily for 6 d to 200 m³ of seawater used for culturing crab larvae. Initial bacterial density in the crustacean culture water was 10⁶ cells/mL. When bacteria increased to more than 10⁷ cells/mL (at crab larval growth stage zoea II), the protozoan population, primarily flagellates, grew rapidly and reduced bacterial numbers to 10⁶ cells/mL. Among the bacterial assemblages monitored, added PM-4 dominated the bacterial populations, i.e. *Vibrio* spp. numbers decreased or even became undetectable in seawater. Production of crab larvae was greatly increased by adding bacterial strain PM-4 to their culture water.

La souche bactérienne PM-4, isolée dans un bassin de culture de crustacés, a amélioré la croissance de la larve du crabe *Portunus trituberculatus* et a inhibé la croissance de *Vibrio anguillarum* dans l'eau de mer. Les bactéries de la souche PM-4 ont été cultivées en grande quantité et ont été ajoutées chaque jour pendant 6 jours à 200 m³ d'eau de mer utilisée pour la culture des larves de crabe. La densité de bactéries initiale dans l'eau de culture des crustacés était de 10⁶ cellules/mL. Lorsque la densité bactérienne a été augmentée à plus de 10⁷ cellules/mL (au stade de développement larvaire zoé II), la population de protozoaires, constituée pour l'essentiel de flagellés, a rapidement augmenté et a réduit la densité bactérienne à 10⁶ cellules/mL. Parmi les associations de bactéries étudiées, les bactéries de la souche PM-4 ajoutées ont dominé les populations bactériennes; en d'autres termes, la population de *Vibrio* spp. a diminué jusqu'à devenir parfois indétectable dans l'eau de mer. La production de larves de crabe a été grandement accrue par l'ajout de bactéries de la souche PM-4 dans leur eau de culture.

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In June 1985 at Tamano Station of the Japan Sea Farming Association, which has been producing millions of larvae of the crab *Portunus trituberculatus* every year since the 1960's, a large proportion of several-day-old larvae was infected with *Vibrio* spp. The bacteria proliferated inside the larval body and 30–40% of the larvae died within a day. Use of antibiotics to repress bacterial growth was ineffective; by the morning following treatment, almost all the larvae were floating near the surface and died later that day. Inside the dead bodies, mycelia of the fungus *Haliphthoros* sp. were very numerous. This same sequence of events was experienced repeatedly, an unsolved problem for crab culture (Muroga et al. 1989).

At the National Research Institute of Aquaculture, Japan, we have been trying to isolate bacterial strains that promote the growth of crustacean larvae (Maeda and Liao 1992). Bacteria were required that would repress the growth of other pathogenic bacteria, especially *Vibrio* spp., but would not kill or inhibit useful microalgae. In this paper, we report the isolation and culture of such a useful bacterium which, when added in large quantities to cultures of *P. trituberculatus* larvae, improves growth and protects them from pathogens without the use of antibiotics.

Materials and Methods

Cultivation of Matured Crab

Spawning females were kept in a container of 2 metric tons, with water depth of 30 cm, a sand mat, and circulating seawater (filtered with sand with grain diameter of about 400 µm) at 25°C. Matured females were transferred from this tank to a 700-L container having gentle aeration about 24 h before their eggs hatched into zoea I.

Seawater

Seawater used for crab culture (200 m³) was filtered through sand (average grain diameter 400 µm) and sterilized with sodium hypochlorite (50 mg/L) before we added the bacterium. This procedure, which eliminated almost all naturally occurring bacteria, was followed by neutralization with sodium thiosulfate at the beginning of the experiment.

Isolation of Bacteria and Yeast

Seawater samples were serially diluted and 0.1 mL of each dilution was spread on plates of 2216E marine agar (Difco Co.

TABLE 1. Survival and moulting rates of the larvae of a crab *P. trituberculatus* in the presence of bacteria and yeasts.

Strains ^a	Microorganism	Survival rate (%)		Activity ^b
		17 h	24 h	
F-1	Yeast	13	0	0
F-2	Bacteria	38	38	0.33
F-3	Bacteria	75	75	0.67
F-4	Yeast	67	11	0.17
F-5	Yeast	44	11	0.25
PM-4	Bacteria	78	78	1.00
<i>B. subtilis</i>	Bacteria	33	33	0.30
Control		50	45	0.66

^aBacterial cells were added to prawn culture water with the diatom *Navicula* sp. (10^4 cells/mL).

^bRatios of the crab larvae that showed phototaxis among the living individuals after 17 h of hatching out.

TABLE 2. Vibriostatic activity of strain PM-4. *Vibrio anguillarum* was inoculated after the preincubation of PM-4 and incubated for 15 d before measurement of its colony width. The reference experiment was done without inoculation of PM-4.

Preincubation period (d)	Colony width (mm)
1	7
3	7
6	5
11	4
Reference	9

Ltd., USA) and incubated for 7 d at 25°C. Colonies were selected and checked for purity on another plate and kept at 25°C for further use.

Cultivation of Bacteria

Bacteria to be added to crab-rearing water were cultured for 4 d in the following medium (all products were from Difco Labs.): Bacto-peptone, Bacto-malt extract, Bacto-yeast extract, and Bacto-soytone (0.05, 0.1, 0.05, and 0.1% (w/v) in seawater, respectively) at pH 7.6 and 25°C.

Addition of Organisms

To 200 m³ of seawater used for rearing the crab larvae, 15 L of the bacterial culture solution was added once every day for 7 d, which resulted in initial bacterial concentrations of about 10^6 cells/mL. Crab larvae (28 000 ind./m³), diatoms (1200 cells/mL), and rotifers (5000 ind./L) were added to culture water on the first day of the experiment.

Counts of Microorganisms

Bacteria and flagellated protozoa were stained with acridine orange and counted directly with an epifluorescent microscope using the methods of Zimmermann and Meyer-Reil (1974) and Hobbie et al. (1977). Single-cell algae were counted without staining.

Plate counts of heterotrophic bacteria and *Vibrio* spp. were done as described by Maeda and Taga (1974) using 2216E marine agar and Drigalski agar (BTB agar, Eiken Co. Ltd., Japan), respectively.

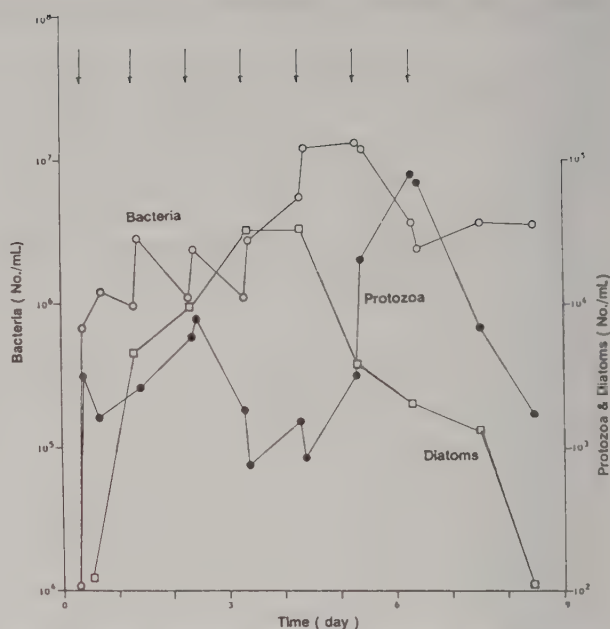


FIG. 1. Numbers of bacteria, protozoa, and diatoms during the culture of crab larvae. Arrows indicate the addition of bacterial culture.

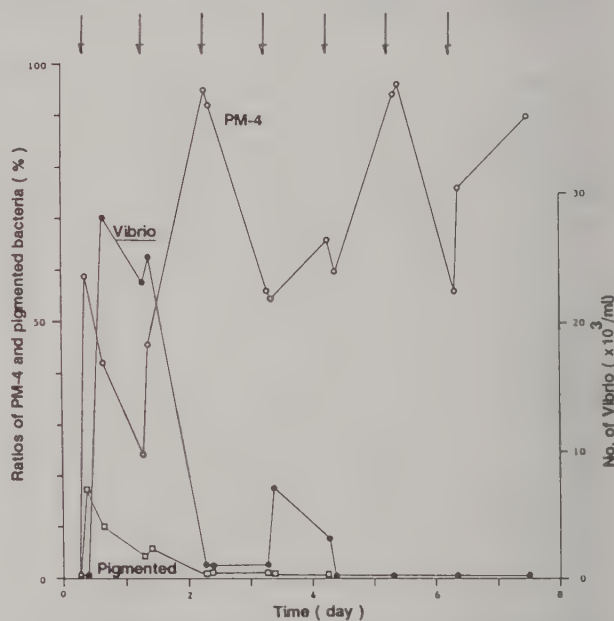


FIG. 2. Fluctuation of PM-4 added, *Vibrio* spp., and pigmented bacteria during the culture of crab larvae. Arrows indicate the addition of bacterial culture.

Determination of Vibriostatic Activity

Two rectangular smears of bacteria to be tested (4 cm in length with a 3-cm gap between smears) were made on a plate of 2216E marine agar, and a 2-cm-long rectangular smear of *Vibrio anguillarum* was made between the two larger smears. Vibriostatic activity was determined in terms of the width of the smear of *V. anguillarum*, i.e. the bacterium for testing was incubated for the periods shown in Table 2. *Vibrio anguillarum* was inoculated between the two smears and incubated for 15 d before measurement. The width of the *V. anguillarum* smear

TABLE 3. Survival rates and production of crab larvae (*P. trituberculatus*) with (biocontrol) and without bacterial strain PM-4 in culture water (1990).

Experiment	Method	Number of larvae ($\times 10^4/200 \text{ m}^3$)					Survival rate (%)	Final production (ind./m ³)
		Z _I	Z _{III}	Z _{IV}	M _I	C _I		
1	Without bacteria	432	392	317	121	11	2.5	550
2	Biocontrol	387	383	281	168	100	25.8	5 000
3	Without bacteria	489	437	402	209	—	—	0
4	Biocontrol	503	457	457	257	78	15.5	3 900
5	Without bacteria	435	435	435	350	192	44.1	9 600
6	Without bacteria	455	433	364	—	—	—	0
7	Biocontrol	455	455	377	291	170	37.4	8 500
8	Without bacteria	538	450	419	—	—	—	0
9	Without bacteria	518	464	457	300	88	17.0	4 400
10	Biocontrol	421	397	395	392	240	57.0	12 000
11	Without bacteria	455	407	349	—	—	—	0
12	Without bacteria	489	376	368	247	—	—	0
13	Biocontrol	422	405	376	226	60	14.2	3 000
14	Biocontrol	442	402	434	306	9 ^a	2.1	465
15	Without bacteria	482	482	381	—	—	—	0
16	Biocontrol	427	413	330	276	160	37.5	8 000
Total	Biocontrol	3057				831	27.2	
	Without bacteria	4263				292	6.8	

^aDecrease by devouring one another.

should be smaller than that of the control plate, if the bacteria tested indeed repress *V. anguillarum*.

Preliminary Experiments to Determine Survival Rates of Crab Larvae with Bacteria and Yeast

Purified bacteria and yeast were inoculated into crab culture water in 1-L containers at a concentration of 10^8 cells/mL. *Portunus* larvae and the diatom *Navicula hustediana* were added to a concentration of 200 ind./L and 10^4 cells/mL, respectively. Survival rates of larvae were measured at 17 and 24 h after the experiment started (Table 1) by counting the number of individuals that swam towards a light source held just outside the vessel.

Results

Several bacterial and yeast strains, including *Bacillus subtilis*, accelerated mortality compared with a diet of diatoms alone (Table 1). On the other hand, bacterial strains F-3 and PM-4 enhanced the survival rate of larvae. Strain F-3 showed increased survival only a little above controls without bacteria. Strain PM-4 was clearly a useful microbe for rearing the crab larvae. This bacterium also repressed the growth of *V. anguillarum* (Table 2).

Addition of PM-4 increased the bacterial density in the culture water to 10^6 cells/mL (Fig. 1) despite active feeding by the zoea I crab larvae (Maeda 1986). When the bacterial density increased to more than 10^7 cells/mL, following decreased bacterial feeding by the zoea II larvae, the protozoan population, primarily flagellates, grew rapidly and reduced the bacteria to about 10^6 cells/mL (Fig. 1).

Among the bacterial populations occurring in culture water of the crab larvae, the numbers of *Vibrio* spp. and pigmented bacteria decreased when PM-4 was added. In particular, there was a negative correlation between PM-4 and *Vibrio* spp. (Fig. 2). In larvae culture water, the numbers of *Vibrio* spp. decreased markedly when the ratio of PM-4 reached more than 60% of the total heterotrophic bacterial population (Fig. 3).

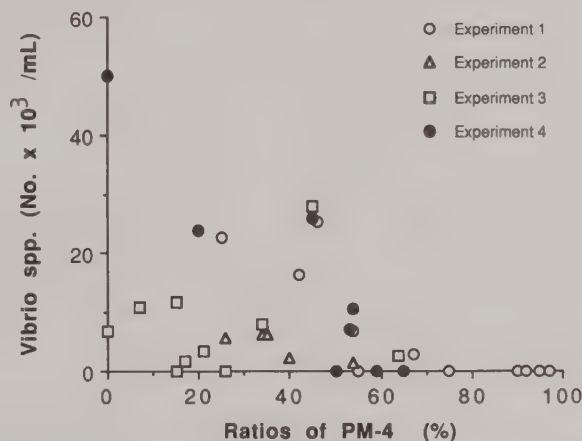


FIG. 3. Ratios of PM-4 of the total heterotrophic bacteria versus numbers of *Vibrio* spp. in the crab culture experiments.

In 1990, we extended our study to larval grow-out using the same experimental procedure as above but at a much larger scale. In seven trials, survival rates of crab larvae in a 200-m³ container was 27.2% (mean value) when bacterial strain PM-4 was added. In six of nine trials in which strain PM-4 was not added, no larvae were grown out, resulting in an average survival rate of only 6.8% (Table 3). A similar result was obtained in 1991 (Table 4).

Discussion

Suzuki et al. (1990) counted numbers of heterotrophic bacteria and *Vibrio* spp. using the plate count method in rearing container water of *Portunus* larval crab to which the strain PM-4 was not added. The heterotrophic bacteria ranged from 10^4 to 10^5 cells/mL during stages zoea I to crab I. *Vibrio* spp. accounted for 15.3, 3.0, 12.7, 15.8, 9.2, and 0.5% of the total bacteria at zoea I, zoea II, zoea III, zoea IV, megalopa, and crab I, respectively.

The term "biocontrol" is becoming familiar in agricultural

TABLE 4. Survival rates and production of crab larvae (*P. trituberculatus*) with (*biocontrol*) and without bacterial strain PM-4 in culture water (1991).

Experiment	Method	Number of larvae ($\times 10^4/200 \text{ m}^3$)					Survival rate (%)	Final production (ind./m ³)
		Z _I	Z _{III}	Z _{IV}	M _I	C _I		
1	Without bacteria	357	390	313	364	234	65.5	11 700
2	<i>Biocontrol</i>	435	502	415	312	175	40.2	8 750
3	Without bacteria	531	430	403	—	—	—	0
4	Without bacteria	439	367	339	201	117	26.7	5 850
5	<i>Biocontrol</i>	473	347	317	154	145	30.7	7 250
6	Without bacteria	475	434	226	—	—	—	0
7	<i>Biocontrol</i>	440	304	322	131	95	21.6	4 750
8	Without bacteria	529	393	384	—	—	—	0
9	Without bacteria	558	528	552	—	—	—	0
10	<i>Biocontrol</i>	508	405	390	264	234	57.8	11 700
11	<i>Biocontrol</i>	555	566	577	318	216	55.0	10 800
					107	89 (divided into two)		4 450
Total	<i>Biocontrol</i>	2411				954	39.6	
	Without bacteria	2889				351	12.1	

science to denote commercial success in controlling the ubiquitous crown gall disease by using an avirulent strain of *Agrobacterium tumefaciens* (also referred to as *A. radiobacter*) (Kerr 1972, 1980). The health of organisms in nature depends primarily upon their inherent resistance to microbial invasion and the biological equilibrium between competing beneficial and deleterious microorganisms at the interface of the organism as mediated by the environment. In aquaculture, some microbes play a major role in this equilibrium in their interactions with various pathogenic agents. In the presence of bacterial strain PM-4, the densities of *Vibrio* spp. and pigmented bacteria were repressed. The latter is also significant because pigmented bacteria tend to inhibit the growth of the larvae (Maeda and Hino 1991).

Two possibilities exist to explain why the growth of *Vibrio* spp. was repressed by PM-4: (1) production of vibriostatic reagents by PM-4 and (2) "niche competition" between zymogeneous bacteria and PM-4.

We have observed similar bacterial densities of about 10^5 – 10^6 cells/mL in several seawater areas including the open Pacific Ocean and eutrophicated coastal areas. The difference in the two areas is the number of protozoa that repress the increase of bacterial populations beyond about 10^6 cells/mL (Maeda and Nogami 1989). This phenomenon means that even in an aquaculture ecosystem the stable maximum density of bacteria seems to be about 10^6 cells/mL. In this research, protozoan populations grew rapidly and reduced bacterial densities to around 10^6 cells/mL, shortly after they had increased to more than 10^7 cells/mL (Fig. 1). We think bacterial feeding by protozoa was intense, since we kept adding the bacterial strain to the water and yet the bacteria remained at about 10^6 cells/mL (Fig. 1).

In the experiments not involving addition of strain PM-4, survival rates of larvae from zoea I to zoea IV were high and larvae were not always infected with pathogenic microbes; however, the larvae died on reaching megalopa I in some experiments (Tables 3 and 4). These data suggest that PM-4 might improve the physiological state of the larvae by serving as a nutrient source during growth.

This bacterium showed the following taxonomic characteristics: a negative Gram staining, no production of budding cells, negative fermentation test, positive motility, peritrichous flagella, and utilization of glucose as substrate. Based on these tests,

this bacterium seems to be similar to the genus *Pseudomonas* or *Deleya*, but the composition of ubiquinone was very different from that of *Pseudomonas* and *Deleya*, i.e. ubiquinone 11 dominated in the total ubiquinone fraction in PM-4. From these data, this bacterium will soon be registered as a new genus and species.

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References

- HOBBIE, J. E., R. J. DALEY, AND S. JASPER. 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33: 1225–1228.
- KERR, A. 1972. Biological control of crown gall: seed inoculation. *J. Appl. Bacteriol.* 35: 493–497.
- . 1980. Biological control of crown gall through production of agrocin 84. *Plant Dis.* 64: 24–30.
- MAEDA, M. 1986. Live feeds — bacteria, p. 157–169. *In* A. Kawai [ed.] *Microbiological aspects in aquaculture*. Koseisha-Koseikaku, Tokyo.
- MAEDA, M., AND A. HINO. 1991. Environmental management for mass culture of the rotifer, *Brachionus plicatilis*. *In* W. Fulks and K. L. Main [ed.] *Rotifer and microalgae culture systems*.
- MAEDA, M., AND I. C. LIAO. 1992. Effect of bacterial population on the growth of a prawn larva. *Penaeus monodon*. *Bull. Natl. Res. Inst. Aquacult.* 21: 25–29.
- MAEDA, M., AND K. NOGAMI. 1989. Some aspects of the biocontrolling method in aquaculture, p. 395–398. *In* S. Miyachi, I. Karube, and Y. Ishida [ed.] *Current topics in marine biotechnology*. Japanese Society of Marine Biotechnology, Tokyo.
- MAEDA, M., AND N. TAGA. 1974. Occurrence and distribution of deoxyribonucleic acid-hydrolyzing bacteria in sea water. *J. Exp. Mar. Biol. Ecol.* 14: 157–169.
- MUROGA, K., K. SUZUKI, N. ISHIBASHI, AND K. NOGAMI. 1989. A vibriosis occurring in zoeal larvae of swimming crab *Portunus trituberculatus*. *Suisanzoshoku* 37: 133–141.
- SUZUKI, K., K. MUROGA, K. NOGAMI, AND K. MARUYAMA. 1990. Bacterial flora of cultured swimming crab (*Portunus trituberculatus*) larvae. *Fish Pathol.* 25: 29–36.
- ZIMMERMANN, R., AND L. A. MEYER-REIL. 1974. A new method for fluorescence staining of bacterial populations on membrane filters. *Kiel. Meer-esforsch.* 30: 24–27.

Factors Limiting Primary Productivity in Lake Ontario Tributaries Receiving Salmon Migrations

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Many tributary streams of Lake Ontario have become important spawning habitats for introduced Pacific salmon. We examine how phosphorus released through salmon decomposition and other environmental factors potentially limit primary productivity in two Lake Ontario tributaries in New York State. Contribution of phosphorus (measured as total phosphorus) from salmon carcasses, computed as the percentage of the estimated phosphorus discharged from the stream, was very low ($<1\% \cdot \text{yr}^{-1}$), but of modest importance ($>50\% \cdot \text{d}^{-1}$) during restricted periods in the spring. Experimental results from three stream sites demonstrated that phosphorus, which is present naturally at high concentrations, is not limiting primary productivity during the period that the salmon are in the streams. Finally, results from an analysis of 15 free-water oxygen studies indicate that the streams are productive (gross primary production $0.9\text{--}10.2 \text{ g O}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) and that light appears to limit stream primary productivity more than nutrients. We conclude that salmon migrations are unlikely to substantially increase the rate of primary productivity in these already fertile streams.

Bon nombre de tributaires du lac Ontario sont devenus des habitats de fraye importants pour le saumon du Pacifique qui y a été introduit. Nous examinons de quelle manière le phosphore libéré par la décomposition du saumon et d'autres facteurs environnementaux limitent potentiellement la productivité primaire dans deux tributaires du lac Ontario dans l'État de New York. La contribution du phosphore (mesuré en phosphore total) provenant des carcasses de saumon, calculée en pourcentage de la quantité de phosphore rejetée par le cours d'eau, est très faible ($< 1\% \cdot \text{an}^{-1}$), mais d'importance modeste ($> 50\% \cdot \text{d}^{-1}$) durant des périodes limitées au printemps. Les résultats expérimentaux de trois stations sur des cours d'eau ont démontré que le phosphore, qui est présent à l'état naturel en concentration élevée, ne limite pas la production primaire durant la période pendant laquelle les saumons peuplent les cours d'eau. Enfin, les résultats d'une analyse de quinze études sur l'oxygène libre dans l'eau indiquent que les cours d'eau sont productifs (production primaire brute comprise entre $0,9$ et $10,2 \text{ g O}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) et que la lumière semble limiter la productivité primaire plus que les éléments nutritifs. Nous concluons qu'il est peu vraisemblable que les migrations du saumon accroissent de manière importante le taux de productivité primaire dans ces cours d'eau déjà fertiles.

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Salmon migrations are thought to be important agents in resupplying nursery streams with phosphorus and thus increasing rates of primary productivity in streams and downstream lakes. The hypothesis is based on the premise that nutrients released from the decaying carcasses become available to nutrient-limited algal communities. Several researchers have investigated the role of migratory fishes as a nutrient source or flux in streams (Hall 1972; Richey et al. 1975; Durbin et al. 1979; Johnson and Ringler 1979) and some have focussed on the role of salmon in the productivity dynamics of western U.S. streams and lakes (Donaldson 1967; Krohkin 1967; Richey et al. 1975; LeBrasseur et al. 1979; Kline et al. 1990).

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Few data exist on the role of native Atlantic salmon (*Salmo salar*) and introduced Pacific salmon (*Oncorhynchus* spp.) in the productive capacity of eastern waters. In addition, none of the previous studies has quantified the relative contribution of migratory fish to phosphorus exported from the stream while also examining the degree to which phosphorus or perhaps other factors limit stream primary productivity.

We tested this phosphorus-subsidy hypothesis on two Lake Ontario tributary streams which serve as spawning habitat for introduced coho (*O. kisutch*) and chinook salmon (*O. tshawytscha*) (Johnson and Ringler 1981a, 1981b). Specifically, we had three main objectives: (1) to test the hypothesis that salmon carcasses represent a significant flux of phosphorus in the study streams, (2) to test whether nutrients limit primary

productivity in the streams during the spawning period, and (3) to measure seasonal aquatic primary productivity as a function of environmental factors in both streams.

Site Description

The two study streams are located in the Tug Hill region of northern New York (Fig. 1). The forest type is predominately northern hardwoods and hemlock. Little Sandy Creek (LSC) drains a significantly larger watershed (6234 ha) than Orwell Brook (OB) (4836 ha) and has more land area denuded of forests (60%) compared with OB (40%) (estimated from U.S. Geological Survey (USGS) topographic maps). Many of the dairy farms, the main form of agriculture in the area, have been abandoned and are gradually converting back to forest cover. These watersheds are underlain by sedimentary rocks covered in most places by 1.5–12 m of glacial deposits (Miller et al. 1989). The growing season is short (135 d) and the winters are severe (up to 500 cm of snow per year). These streams received a run of native Atlantic salmon in the past, but the building of dams, pollution, and overfishing contributed to the extirpation of the population by the turn of the century (Webster 1982). Coho and chinook salmon were successfully introduced into the system during the 1960's (Parsons 1973) and have established spawning populations in many tributaries of eastern Lake Ontario. The two study streams were selected in part because

of early observations of relatively large numbers of returning adult salmon and extensive evidence of natural reproduction (Johnson and Ringler 1981a, 1981b; Wisniewski 1990; N. H. Ringler and J. G. Kennen, unpubl. data).

Methods

The overall approach of the study was to quantify nutrient input into the streams through salmon migrations, test for nutrient limitation, and measure rates of in situ primary productivity in the two study streams during an integrated sampling period from fall 1987 to fall 1989. All the components of the ecosystem analyzed in the study are displayed in the form of an energy and material flow diagram (Fig. 2).

Phosphorus Dynamics

To quantify the amount of phosphorus introduced through a salmon migration, we estimated the mass of decomposing carcasses resulting from a year's migration. Salmon carcass surveys were conducted in fall 1987 and 1988 (approximately October to December) on OB and LSC. Ground surveys were conducted by a pair of observers walking along each bank at approximately 10-d intervals. Carcasses located in previous surveys were identified with plastic streamer tags attached to the lower mandible. The counts were minimum estimates because some salmon were hidden behind log jams or amidst streamside

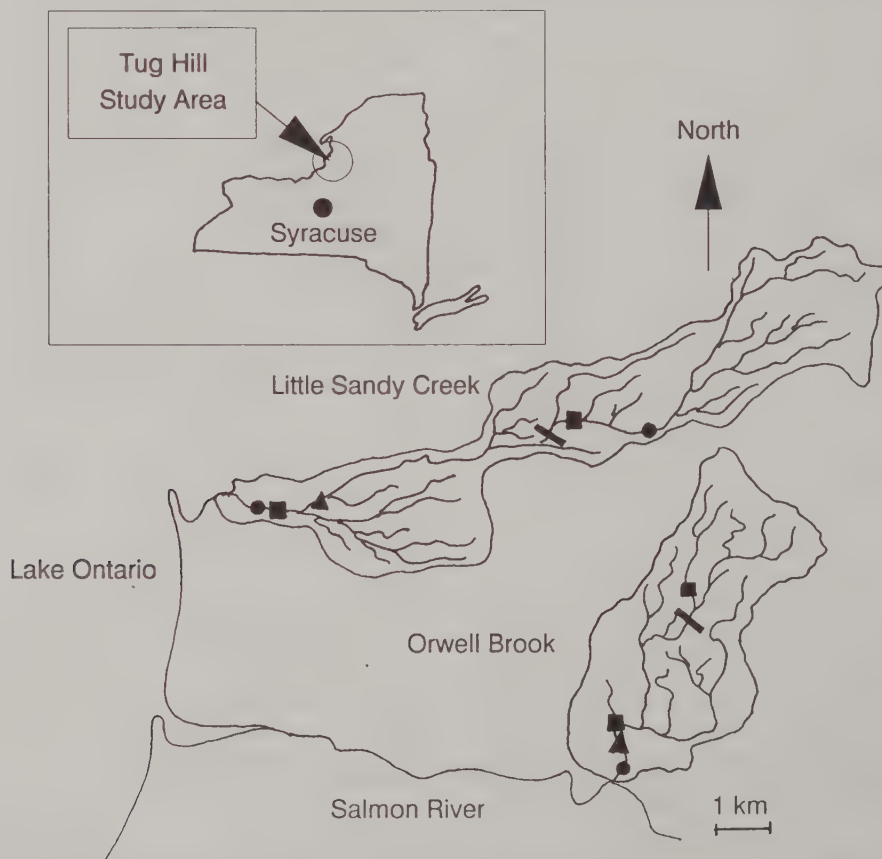


FIG. 1. Study area, showing the sites for measurement of phosphorus concentration and water discharge (●), the nutrient limitation experiments (■), and primary productivity measurements (▲). The approximate upstream limit of the salmon migration during the two years studied is also identified (bar).

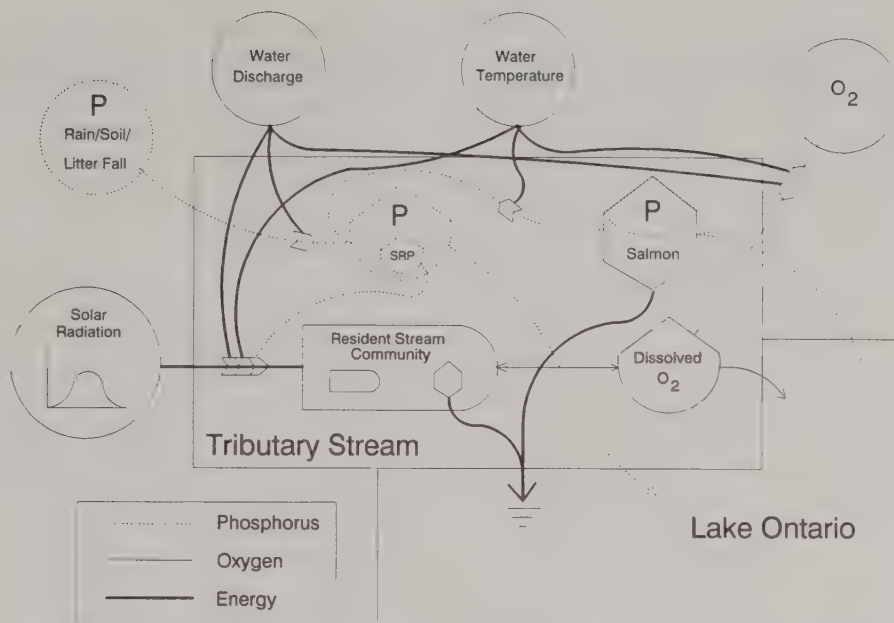


FIG. 2. Material and energy flows quantified for the Lake Ontario tributary streams described in this study. Symbols as in Odum (1983).

vegetation. We used Donaldson's (1967) estimate of the phosphorus content for sockeye salmon (*O. nerka*) (0.364% of the wet weight) to approximate the value for Lake Ontario Pacific salmon. Mass of carcasses was converted to kilograms of body phosphorus by multiplying the wet weight by this conversion factor.

To estimate phosphorus release from salmon, we measured decomposition rates of salmon in the field from November 1987 through May 1988. Dead mature Pacific salmon (20 coho and chinook) obtained from Beaverdam Brook in Altmar, NY, were weighed and placed within microenclosures constructed from 1.3-cm square mesh galvanized hardware cloth. This design allowed access of potential invertebrate decomposers, yet prevented tampering by vertebrate scavengers. These cages were placed along OB in aquatic and terrestrial sites to assess any site-specific variation in decomposition. Wet weight was measured periodically over a 5-mo period. Access to the sites was limited in January and February due to snow conditions.

The forms of phosphorus released from the carcasses during the decomposition study were measured by immersing the hardware cloth enclosures into water held in a large plastic container and allowing phosphorus to be released from the salmon into solution over a 20-min period. This was carried out on three separate dates (November, December, and February) on a randomly selected aquatic and terrestrial carcass. Water samples, taken before and after carcass immersion, were analyzed to estimate relative proportions of available (soluble reactive phosphorus (SRP)) and nonavailable (total phosphorus (TP) minus SRP) forms of phosphorus in the ambient stream water and to characterize the phosphorus released during the 20-min incubation.

Estimated annual TP export from both watersheds was calculated by summing the product of estimated stream discharge and ambient TP concentration on each day over an entire water year (October 1 to September 31). Instantaneous stream discharge was measured at the mouth of each stream (Fig. 1) by

measuring cross-sectional area and mean water velocity using a Pygmy-Gurley (model 662) flowmeter. A continuous record of daily mean discharge over the two water years was obtained from the USGS gauge station on Sandy Creek at Adams, NY, located approximately 15 km north of LSC. The discharge measurements made on OB and LSC were regressed against discharge recorded on the same day at the Sandy Creek gauge station. A linear regression was successful in relating gauged stream discharge (Sandy Creek) to ungauged study stream discharge (OB and LSC) (r^2 for all eight sites > 0.81 ; $n = 14$ per site). The slopes of the regression lines indicated that the discharge rate of the study streams is approximately one-tenth that of Sandy Creek.

Concentrations of TP in OB and LSC were monitored from November 1987 to July 1989. Water samples were taken at the mouth of both study streams. Water samples were also collected from a headwater station on Little Sandy Creek to characterize the water draining a relatively undisturbed area (Fig. 1). A total of 31 water samples ($n = 10$ at headwater station) were collected over a range of stream discharge including periods during spring runoff. Discharge from the study streams exceeded the range sampled during only 3 d during the two water years analyzed. All water samples were frozen until the day of analysis. TP concentrations were determined on unfiltered samples using the ammonium molybdate technique following persulfate digestion (Murphy and Riley 1962; Strickland and Parsons 1968). Accuracy of laboratory analyses was assessed by analyzing three U.S. Environmental Protection Agency phosphorus standards. All three estimates of TP concentration were within 7% of the concentration of the standard sample.

Nutrient Limitation

Salmon carcasses in this region often overwinter in a frozen condition, and much of the decomposition occurs after snowmelt the following spring (Johnson and Ringler 1979; Fisher

1981). Based on these observations, we conducted nutrient enhancement experiments during spring 1989.

We measured algal growth on artificial substrates (small clay flowerpots filled with nutrient-enriched agar) incubated in the stream (Fairchild and Lowe 1984). Specifications of amount and form of nutrients added to the agar were described in Fairchild and Lowe (1984). In addition, we used an approach similar to that used by Minshall et al. (1991) to test whether the nutrients released from decaying salmon carcasses increase productivity immediately downstream. Clay pots with no added nutrients were placed downstream of decaying fish, where nutrient concentrations were presumably greater than the ambient concentrations in the stream.

Fifteen pots were placed at each of four study sites (upstream and downstream site on each stream) (Fig. 1). Upstream sites on both streams were located above the upstream limit of salmon migration. We chose fairly shallow riffles approximately 0.3 m deep. At each site, 12 of the pots served as experimental nutrient treatments (nitrogen, phosphorus, nitrogen plus phosphorus, carcass nutrients; $n = 3$ per treatment) and the remaining three pots served as a control (no nutrients). The nitrogen, phosphorus, nitrogen plus phosphorus, and control pots were placed in a row perpendicular to the current to prevent contamination between different treatments. Adjacent to these pots, a carcass nutrient experiment was designed by placing pots with no added nutrients 30 cm downstream of a decaying carcass. Each treatment set of three pots was fastened to hardware cloth, and rocks were used to anchor the cloth to the substrate.

The pots were incubated in the streams for 36 d (prior to leaf-out). After 23 d, the pots located at the downstream site on LSC were damaged and displaced by a storm. The pots were removed and replaced with a completely new set that was incubated for 36 d following that date. Because the results of the experiments are displaced in time, the original, undisturbed treatments will be referred to as experiment 1 and the treatments replaced after 23 d as experiment 2. Following the experimental period, the pots were scraped and chlorophyll was extracted with 90% acetone and analyzed spectrophotometrically for chlorophyll *a* and corrected for phaeophytin (APHA 1989). Results are presented as milligrams of chlorophyll *a* per square metre of pot surface.

Comparison between chlorophyll *a* biomass means for treatments at each site was carried out using ANOVA. For the sites that exhibited significant differences at $p < 0.05$, multiple comparisons were conducted between selected individual treatments using Fisher's protected LSD ($p < 0.05$), a test applicable to unequally replicated treatments. All p values are one-sided.

Measurements of solar radiation were obtained from a recording station in Oswego, NY (approximately 45 km southwest of the study area). Average daily water temperatures were obtained from a Ryan model J thermograph placed near the mouth of each stream from April to July 1989. Concentrations of SRP were measured at the four pot sites on four dates during the time of incubation. Samples were collected immediately upstream of the study area to characterize the water passing over the pots. Samples were iced immediately in the field, filtered (0.45- μ m Millipore) within 2 h of collection, and analyzed using the ammonium molybdate technique (Murphy and Riley 1962; Strickland and Parsons 1968).

Primary Productivity

Fifteen 24-h field studies were conducted from April 1988 to November 1989 at a 415-m reach on lower OB and two contiguous 400-m reaches on lower LSC (Fig. 1) to measure in

situ rates of primary productivity. The study reach on OB passes through an agricultural field and is lined with a secondary growth riparian community consisting mostly of willows, hemlock, and alders. This stream reach was characterized as relatively deep (approximate mean depth at base flow = 0.4 m) with a moderate current velocity (base flow approximately $0.2 \text{ m}^3 \cdot \text{s}^{-1}$), with two of seven sections identified as riffles. The upstream and downstream reaches on LSC were divided into nine and 16 sections, respectively. The upstream reach on LSC (referred to as Reach 1) has an open canopy and is shallow (approximate mean depth at base flow = 0.2 m) with all nine sections designated as riffles. The downstream reach (Reach 2) passed through a secondary-growth deciduous forest and is slightly deeper (approximate mean depth at base flow = 0.3 m) with alternating pools and riffles. More detailed physical dimensions for both study sites were quantified under low-flow conditions. The length, average width, and average depth were measured for each section of the study reach. A cross-section velocity profile was measured at the middle of each section with a Pygmy-Gurley (model 662) flowmeter. Cross-sectional channel measurements were carried out on LSC over a range of discharge levels to derive a relationship between stream channel characteristics (average width, depth, and current velocity) and discharge level.

Community metabolism for each study stream was measured using Odum's two-station diel curve technique as outlined in Odum (1956) and Hall and Moll (1975). Dissolved oxygen (DO) was measured using a galvanic probe (YSI model 51). The accuracy of the probe DO determination was assessed by calibrating simultaneously with Winkler titrations in the field. Pooling 35 separate comparisons, the probe measured consistently higher (an average of 6%) than the Winkler technique. All the values obtained from the DO meter were multiplied by 0.94 to compute a corrected DO reading. Solar radiation was measured in an open field adjacent to each study site using a pyroheliometer (measured in units of calories per square centimetre). Attenuation of light by canopy cover was simulated using a sine function to account for incremental loss of light reaching the stream in spring and the increase of light penetration following leaf-fall (Rand 1990). Water temperature was measured with a thermistor.

Estimates of reaeration in the three study reaches were derived from a simple model of stream hydrology. The low-flow physical values measured in the field were transformed to simulate the stream conditions on the day of sampling. This was accomplished by regressing stream width, depth, and current velocity to the discharge at the mouth of the stream measured on the day of sampling. These simulated physical values were used to compute reaeration parameters. The following equation, developed by Negulescu and Rojanski (1969) and later validated by Grant and Skavronck (1980), was selected to compute reaeration coefficients (K_2 , per day):

$$K_2 = 12.29 \cdot (V/H)^{0.85}$$

where V = average stream velocity (m/s) (metres per second) and H = average hydraulic depth (metres).

To estimate gross and net primary productivity, we integrated the area under the DO rate of change curve as in Hall and Moll (1975). Daytime respiration was estimated by connecting the predawn and postdusk rate of change points with a straight line. The integrated areas (grams of oxygen per cubic metre per day) representing gross productivity and respiration were multiplied by the mean depth of the study reach on the day of sampling

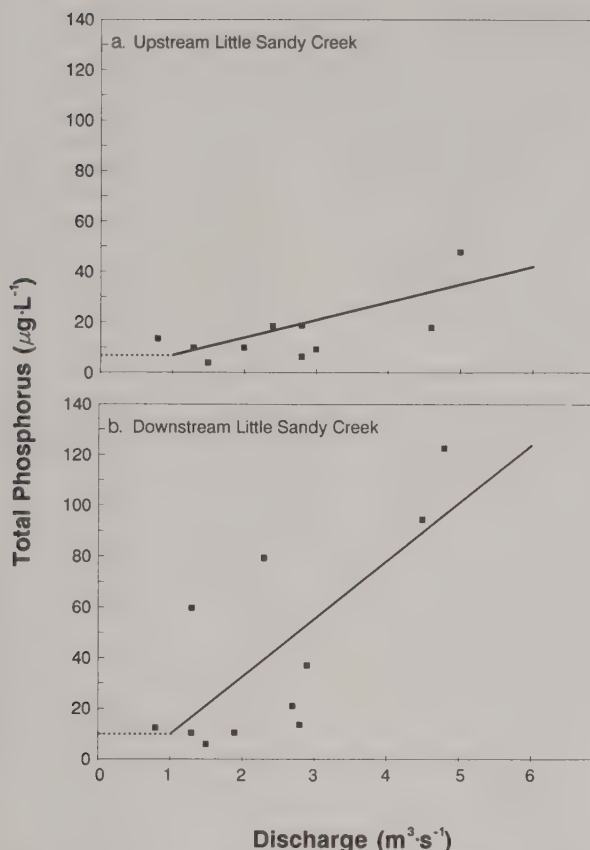


FIG. 3. Regressions relating discharge of study stream to TP concentration measured at two locations on LSC. Slopes from both linear regressions were found to be statistically different from zero ($p < 0.05$). The dotted line represents an approximation used in the model for estimating TP concentration at low stream discharge.

to estimate daily total gross and net primary productivity (grams of oxygen per square metre per day).

Results

Phosphorus Dynamics

A linear model was moderately successful in describing variation in TP concentration versus discharge for both upstream and downstream sites on LSC (Fig. 3). At both sites, concentration of TP increased with an increase in channel discharge ($p < 0.05$). The slope of the regression for the downstream site at OB was not significantly different from zero and the data were used to generate a pooled mean TP concentration (mean = $24.0 \mu\text{g}\cdot\text{L}^{-1}$, SD = 10.3, $n = 10$). This limited response of TP concentration to discharge in OB might be partly explained by the fact that OB has a greater proportion of its watershed under forest cover and thus conserves more nutrients.

In both migratory seasons, LSC had significantly higher carcass mass (4754 kg in 1987, 3484 kg in 1988) than OB (1337 kg in 1987, 396 kg in 1988). A possible explanation is that LSC receives less angling pressure because the stream was closed to fishing to protect the newly reintroduced Atlantic salmon. The salmon generally traveled no more than 10 km up the main stem of OB and 16 km up LSC (Fig. 1). Carcass

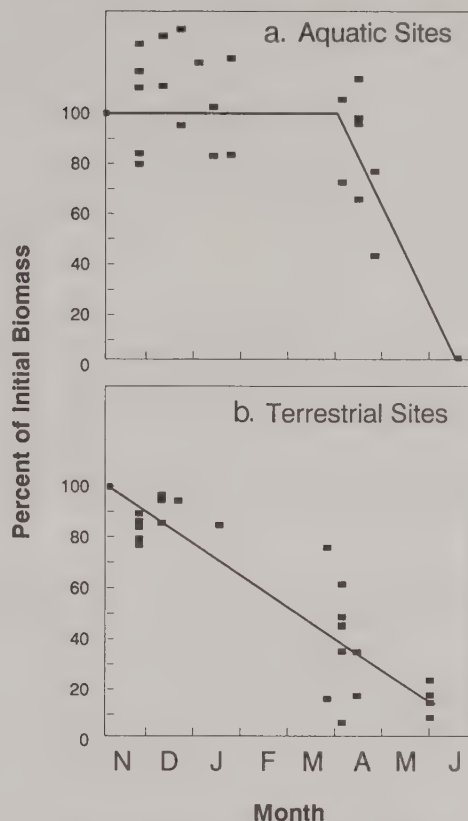


FIG. 4. Percentage of initial mass remaining over time for carcasses incubated in (a) aquatic and (b) terrestrial sites on OB during the fall, winter, and spring of 1987–88.

phosphorus standing crop for a high spawner abundance year (1987) was estimated as 0.9 and $4.8 \text{ kg P}\cdot\text{ha stream surface}^{-1}$ for OB and LSC, respectively (stream surface area estimate includes only the distance of stream traversed by the salmon).

The decomposition process in 1987–88 extended over the winter in both the aquatic and the terrestrial sites (Fig. 4a, 4b). These results are confounded by the fact that the carcasses placed in the stream absorbed water by osmosis and any loss in weight due to decomposition was more than compensated for by an increase in water weight. To correct for this osmotic phenomenon, it was assumed that the aquatic carcasses retained their initial weight from October to March. The decomposition rate was then assumed to proceed linearly from March to the end of May. The data for terrestrial decomposition were fit to a single linear regression (Fig. 4b; $r^2 = 0.87$).

The phosphorus released through salmon decomposition appears to be relatively enriched in SRP compared with the ambient ratio (assessed as micrograms of SRP per litre divided by micrograms of TP per litre). The available fraction of phosphorus released from the carcasses (mean ratio = 0.5, $n = 7$) was larger than the ratio measured for the ambient stream water (mean ratio = 0.4, $n = 7$; t -test, $p < 0.10$).

TP export from LSC was estimated by multiplying the predicted TP concentration (from the regression in Fig. 3) by the daily mean discharge. Because there was no evidence of an effect of discharge on TP concentration in OB, the estimated daily mean discharge from OB was multiplied by the pooled

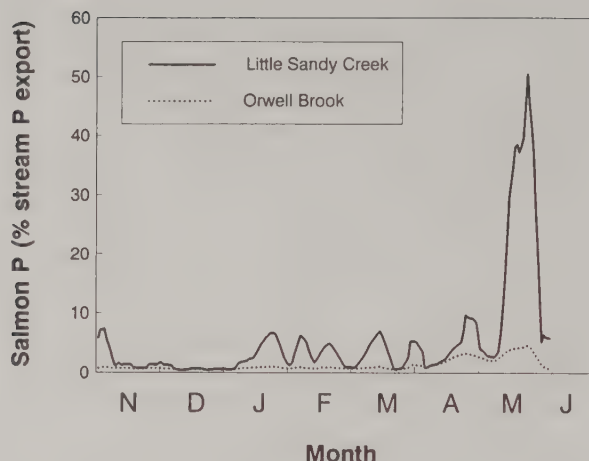


FIG. 5. Simulated carcass phosphorus release in LSC in 1987–88 represented as a percentage of daily TP discharge. Output is represented as a 7-d moving average.

mean TP concentration. The contribution of carcass phosphorus (percent) to the annual stream TP export was estimated to be very small for both streams. The contribution of salmon phosphorus from water year 1987 (October 1 to September 30) to water year 1988 dropped from 1.0 to 0.4% in LSC and from 0.6 to 0.1% in OB as a result of the decrease in spawner abundance coupled with higher export from the watershed due to increased precipitation during water year 1988.

TP export is positively correlated with degree of land use disturbance and annual precipitation. The LSC watershed, with 13% less area in forest, exported an average of 34% more TP per unit of land area than OB. Phosphorus export from LSC increased 27% from a “dry” year ($30.3 \text{ mg} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$) to a “wet” year ($41.1 \text{ mg} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$). OB was less responsive ($14.0\text{--}18.0 \text{ mg} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$) to changes in annual precipitation.

Daily estimates of TP release from carcass decomposition (estimated by multiplying wet weight loss by 0.364%, the proportion of phosphorus in salmon carcass) appeared to be significant during restricted periods of the year (Fig. 5, presented as 7-d moving average). During periods of low discharge during the spring, TP originating from carcass decay in LSC can represent greater than 50% of the amount of TP discharged daily from the stream mouth. Because of lower carcass biomass, this effect in OB was less pronounced.

We developed an historical model to simulate Atlantic salmon carcass decay in LSC occurring under presettlement, oligotrophic conditions. To account for the large numbers of returning Atlantic salmon documented in historical journals (Webster 1982), carcass mass was initialized at twice the maximum standing crop of Pacific salmon measured during this study (undoubtedly a generous estimate), which presumably accounts for the smaller proportion of native Atlantic salmon that die following spawning. The TP versus discharge relationship for the LSC upstream water quality site (Fig. 3a; draining relatively undisturbed areas) was used to represent the functional response of the whole stream (regression: $\text{TP} (\mu\text{g} \cdot \text{L}^{-1}) = 6.36 \cdot \text{discharge} (\text{m}^3 \cdot \text{s}^{-1}) - 0.16$; $r^2 = 0.69$, $n = 10$). This model run resulted in a decrease in annual TP discharge of 63% in LSC. The contribution of carcasses to the annual phosphorus export increased from 1.0% in the nominal run to 5.4% in the oligotrophic, high spawner abundance run.

Nutrient Limitation

These experiments indicated that neither nitrogen or phosphorus is likely to be limiting primary productivity over the period of salmon carcass decomposition. Algal biomass, assessed as milligrams of chlorophyll per square metre of pot surface, ranged from 0.2 to $61.8 \text{ mg} \cdot \text{m}^{-2}$. Phosphorus limitation was evident during the later experiment (experiment 2) at the lower LSC site (Table 1). At that site, algal biomass on the pot with phosphorus was greater than the control treatment ($p < 0.05$). It is unclear why the same positive response was not observed in the pots containing both nitrogen and phosphorus at that site.

There were no significant differences between mean algal biomass for the carcass nutrient treatment and the control at each of the three sites having carcass treatments (Table 1). Results from the ANOVA for the conventional nutrient treatments showed differences ($p < 0.05$) in all but the upper OB site (Table 1). Comparison of treatment means for these three sites showed no evidence of nutrient limitation with the exception of the lower LSC site described above. Nitrogen appeared to have an inhibitory effect on algal growth at the downstream OB site and nitrogen and phosphorus both suppressed algal growth at the upper LSC site (Table 1).

Daily integrated solar radiation during the pot incubation was highly variable, with a mean of $416 \text{ cal} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ during experiment 1 and $449 \text{ cal} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ during experiment 2. Water temperature means were 10.4 and 14.8°C during experiments 1 and 2, respectively, and water temperature was consistently lower (approximately 1.4°C less) in LSC compared with OB. Concentration of SRP measured at all four sites over the incubation period showed no clear trends and variation was minimal (mean = $32.6 \mu\text{g} \cdot \text{L}^{-1}$, SD = 6.9, $n = 16$).

Primary Productivity

Estimates for gross productivity ranged from 0.9 to $10.2 \text{ g O}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, community respiration varied from 0.6 to $14.0 \text{ g O}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, and net productivity ranged from -13.6 to a high of $4.1 \text{ g O}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ (Table 2).

Variations in gross primary productivity (GPP) measured at both sites were best explained by variation in daily integrated solar insolation. We fit a Michaelis–Menten equation to the data and plotted the residuals against cumulative rainfall 3 d prior to sampling, mean stream discharge, mean DO concentration, and mean water temperature. The latter was most successful in explaining variation in the residuals. The data from both streams were fit to a positive slope line reflecting higher rates of productivity at higher ambient temperatures. The resulting regression line, GPP as function of solar radiation (SRAD) and corrected for water temperature, was moderately successful in predicting gross productivity (equation: $\text{GPP} (\text{g O}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}) = 10 \cdot (\text{SRAD} / (150 + \text{SRAD}))$; $r^2 = 0.54$) (Fig. 6).

Community respiration (CR) appears to be primarily of function of water temperature (Table 2). The data were fit to a Q_{10} physiological function and residuals were analyzed against the measured variables listed above. Cumulative rainfall prior to sampling was found to be an important factor in CR in OB ($r^2 = 0.67$). None of these parameters was successful in explaining the residual variation for LSC. The resulting equation was successful in explaining more of the variability in the OB data (equation: $\text{CR} (\text{g O}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}) = 1.0 \cdot 3.0^{(\text{temp}/10)}$, $r^2 = 0.80$).

TABLE 1. Estimates of chlorophyll biomass (mg Chl $a \cdot m^{-2}$ pot surface $^{-1} \pm$ 95% confidence interval) on nutrient diffusing substrates. Sample size per cell is three unless specified. Asterisks represents a significant difference between a given nutrient treatment mean and the control at each site ($p < 0.05$).

Treatment	Experimental site			
	Upstream OB	Downstream OB	Upstream LSC	Downstream LSC
Control	24.1 $\pm$ 16.1	50.8 $\pm$ 25.1	11.2 $\pm$ 7.5	9.3 $\pm$ 6.2
Nitrogen	15.0 $\pm$ 12.2	19.0 $\pm$ 7.0*	2.0 $\pm$ 15.3*(2)	8.7 $\pm$ 4.7
Phosphorus	23.9 $\pm$ 21.1	39.9 $\pm$ 18.1	5.8 $\pm$ 5.2*	16.2 $\pm$ 5.0*
Nitrogen plus phosphorus	11.2 $\pm$ 5.0	31.9 $\pm$ 17.1(2)	1.9 $\pm$ 4.5*	9.4 $\pm$ 4.2
Salmon	36.4 $\pm$ 29.8	34.3 $\pm$ 38.5	—	7.6 $\pm$ 7.2

TABLE 2. Stream primary productivity measurements (GPP) made at OB and LSC. Solar radiation (SRAD) measurements presented are corrected for riparian canopy attenuation as described in the text.

Date	Site	SRAD (cal $\cdot$ cm $^{-1}$)	Mean temp. ($^{\circ}$ C)	Mean [O ₂] (g $\cdot$ m $^{-3}$)	Discharge (m 3 $\cdot$ s $^{-1}$)	GPP (g O ₂ $\cdot$ m $^{-2}$ $\cdot$ d $^{-1}$)	CR (g O ₂ $\cdot$ m $^{-2}$ $\cdot$ d $^{-1}$)
<i>OB</i>							
Apr. 28, 1988		644	9.5	11.2	0.6	2.1	0.6
May 20, 1988		436	16.0	8.0	1.9	9.1	9.3
Sept. 4, 1988		23	17.0	8.8	1.1	2.9	2.5
Sept. 7, 1988		97	14.0	9.9	0.6	3.8	7.3
Oct. 1, 1988		283	15.0	8.9	0.1	6.4	2.2
June 17, 1989		91	16.0	9.0	1.1	2.5	2.2
Nov. 19, 1989		66	3.7	12.8	1.2	2.9	3.6
<i>LSC</i>							
July 14, 1989	1	678	21.5	9.0	0.7	10.2	6.8
July 14, 1989	2	136	20.8	8.9	0.7	0.9	14.0
Aug. 18, 1989	1	543	19.0	9.4	0.7	7.3	5.0
Aug. 18, 1989	2	109	10.0	9.2	0.7	1.1	9.1
Sept. 10, 1989	1	239	21.0	8.3	0.7	10.0	11.3
Sept. 10, 1989	2	48	21.5	8.2	0.7	2.7	7.0
Oct. 29, 1989	1	207	10.0	11.1	—	5.6	5.0
Oct. 29, 1989	2	207	18.9	11.0	—	2.5	0.6

Discussion

Salmon migrating into the two study streams appear to account for only a small fraction ($\leq 1\%$) of the phosphorus exported annually from the watershed, although phosphorus released from salmon decomposition measured as a proportion of daily export can be significant during certain periods in the spring. In addition, the salmon phosphorus released is relatively enriched in the fraction that is biologically available. Although salmon phosphorus can represent a major flux in the spring, the streams do not appear to be nutrient limited during that period. Results from the primary productivity study indicate that light may be a more important limiter of productivity in these streams. Results of the historical Atlantic salmon simulation also indicated that it is unlikely that salmon carcasses were a significant annual contributor of phosphorus to these streams in the past.

It is important to note that other pathways may be more important in subsidizing ecosystem productivity in these streams. Eggs deposited in the streams after spawning may serve as an important energy source for resident fish (J. G. Kennen and N. H. Ringler, unpubl. data), and aquatic fungi and invertebrates also appear to use the carcasses as a source of energy,

a phenomenon noted also by Minshall et al. (1991) in an Idaho stream.

A number of important assumptions were made in the process of estimating phosphorus discharge. A hysteresis response in the TP versus discharge relationship in streams has been observed in a number of studies, with the rising limb of the hydrograph characterized by higher concentrations of TP relative to the falling limb (Meyer and Likens 1979; Rigler 1979). It was assumed in the model that TP concentration was a linear function of discharge. This assumption was necessary because no short-term time series data on TP concentration during storm events were collected from which we could derive a hysteresis response curve. We assumed that the linear regression model accurately represents TP dynamics during periods of fluctuating discharge.

We also assumed that the relationship between TP concentration and stream discharge is independent of season. Although others have found distinct seasonal patterns in this relationship (e.g. Meyer and Likens 1979), we pooled our data, and site-specific functional response curves were used for each stream. Also of concern is the high variance about the regression line for this relationship at the downstream site on LSC (see Fig. 3b). Although this does contribute to the uncertainty of the TP export

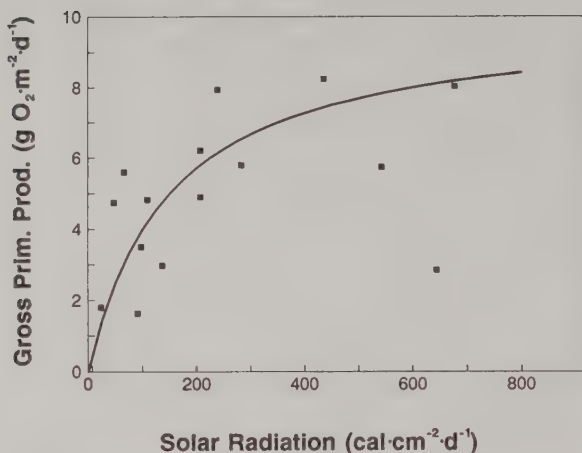


FIG. 6. Gross primary productivity plotted as a function of daily total solar radiation and corrected for water temperature. The data from both streams were pooled and fit to a Michaelis-Menten equation ($r^2 = 0.54$). Solar radiation is corrected for canopy attenuation as described in the text.

estimates, the model conclusions appear to be robust given the results of the Atlantic salmon simulation described in the results section.

Many pathways of dispersal of fish tissue have been documented, including consumption by blowfly larvae and terrestrial vertebrate scavenging (Fisher 1981; Cederholm and Peterson 1985; Cederholm et al. 1989). Discharge regime is important in moving carcasses between aquatic and terrestrial sites and, under higher levels of discharge, is even likely to flush carcasses completely from the stream (Richey et al. 1975). We assumed that all carcass TP derived from the carcass survey data contributed to dissolved and suspended TP in the water column, and therefore, carcass TP contribution in all cases should be considered a maximum.

Pacific salmon carcasses contribute significant amounts of phosphorus to rivers and lakes of the western United States. In fact, Donaldson (1967) concluded that the TP originating from the 1965 escapement of sockeye salmon into Iliamna Lake in Alaska was 1.37 times greater than the amount of phosphorus entering the lake that year from tributaries and the atmosphere and formed 68% of the total phosphorus in the lake water. In addition, Krohkin (1967) found that nearly 40% of the TP income in Lake Dalneer was supplied by the dead bodies of spawned-out sockeye salmon.

Why is the annual contribution of carcass phosphorus in these eastern streams so small? One possibility is that the density of returning adult salmon is significantly less in these eastern streams compared with the number of returning salmon observed in the western states. The only meaningful comparison that can be made is to the kokanee salmon (*O. nerka*) migration of Taylor Creek in Nevada measured by Richey et al. (1975). The range of discharge of Taylor Creek ($0.1\text{--}6\text{ m}^3\cdot\text{s}^{-1}$) is comparable with that observed for our study streams ($0.1\text{--}11.0\text{ m}^3\cdot\text{s}^{-1}$). Richey et al. (1975) estimated that 44.6 kg of phosphorus was introduced as a result of a large migration in 1971, which compared with only 17.3 kg of phosphorus for the largest migration measured in LSC in 1987. A second reason for the geographical differences may be that western streams are intrinsically more oligotrophic and hence export less TP over an annual cycle. Donaldson (1967) calculated a mean TP

concentration (volume-weighted) of less than $8\text{ }\mu\text{g}\cdot\text{L}^{-1}$ in tributaries of Iliamna Lake, significantly less than those observed in this study.

Both the nutrient limitation study and the primary productivity analysis revealed that TP release from salmon decay in OB and LSC takes place mostly during periods when phosphorus is not likely to be limiting primary productivity. Our nutrient limitation results indicate that salmon migrations do not play an important role in maintaining observed levels of productivity in the two study streams. The concentration of SRP measured during the experiment was generally high compared with other streams which are nutrient limited (e.g. Richey et al. 1975; Elwood et al. 1981; Peterson et al. 1985; Lowe et al. 1986; Pringle 1987). The differences in productivity observed between the two streams can not be readily explained by differences in the concentration of SRP.

The primary productivity study indicated that light limitation is more important in reaches of the stream flowing under canopy cover and in all parts of the stream during periods when light intensity is low and photoperiod is short. The later nutrient limitation experiment (experiment 2) demonstrated nutrient limitation during late spring and appeared to support this hypothesis. These streams are relatively productive (gross productivity = $0.86\text{--}10.19\text{ g O}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$), especially when compared with streams studied in Oregon and British Columbia (gross productivity = $0.1\text{--}2.5\text{ g O}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, from Naiman and Sedell 1980).

The magnitude of salmon phosphorus input to these streams is likely to increase dramatically over the next decade following the proposed changes in fishing regulations promulgated by the New York Department of Environmental Conservation. The new regulations will prohibit snagging Pacific salmon in Lake Ontario tributary streams by 1994, thus allowing more fish to complete their migration to the spawning grounds, where salmon carcass density will likely increase. This change in fishery regulations could serve as a natural experiment to further test the hypothesis described in this study.

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References

- AMERICAN PUBLIC HEALTH ASSOCIATION (APHA), AMERICAN WATERWORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1989. Standard methods for the examination of water and wastewater. 17th ed. American Public Health Association, Washington, DC.
- CEDERHOLM, C. J., D. B. HOUSTON, D. L. COLE, AND W. J. SCARLETT. 1989. Fate of coho salmon (*Oncorhynchus kisutch*) carcasses in spawning streams. Can. J. Fish. Aquat. Sci. 46: 1347–1353.
- CEDERHOLM, C. J., AND N. P. PETERSON. 1985. The retention of coho salmon (*Oncorhynchus kisutch*) carcasses by organic debris in small streams. Can. J. Fish. Aquat. Sci. 42: 1222–1225.
- DONALDSON, J. R. 1967. The phosphorus budget of Iliamna Lake, Alaska as related to the cyclic abundance of sockeye salmon. Ph.D. dissertation, University of Washington, Seattle, WA. 141 p.

- DURBIN, A. G., S. W. NIXON, AND C. A. OVIATT. 1979. Effects of the spawning migration of the alewife, *Alosa pseudoharengus*, on freshwater ecosystems. *Ecology* 60: 8–17.
- ELWOOD, J. W., J. D. NEWBOLD, A. F. TRIMBLE, AND R. W. STARK. 1981. The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. *Ecology* 62(1): 146–158.
- FAIRCHILD, G. W., AND R. L. LOWE. 1984. Artificial substrates which release nutrients: effects on periphyton and invertebrate succession. *Hydrobiologia* 114: 29–37.
- FISHER, M. 1981. Faunal decomposition of salmon carcasses in terrestrial and aquatic sites in central New York. M.S. thesis, SUNY College of Environmental Science and Forestry, Syracuse, NY. 64 p.
- GRANT, R. S., AND S. SKAVRONECK. 1980. Comparison of tracer methods and predictive equations for determination of stream-re-aeration coefficients on three small streams in Wisconsin. U.S. Geol. Surv. Water Resour. Invest. 80-19: 36 p.
- HALL, C. A. S. 1972. Migration and metabolism in a temperate stream ecosystem. *Ecology* 53(4): 585–604.
- HALL, C. A. S., AND R. MOLL. 1975. Methods of assessing aquatic primary production. In H. Lieth and R. H. Whittaker [ed.] Primary productivity of the biosphere. Springer-Verlag, New York, NY.
- JOHNSON, J. H., AND N. H. RINGLER. 1979. The occurrence of blow fly larvae (Diptera: Calliphoridae) on salmon carcasses and their utilization as food by juvenile salmon and trout. *Great Lakes Entomol.* 12(3): 137–140.
- 1981a. Natural reproduction and juvenile ecology of Pacific salmon and steelhead trout in tributaries of the Salmon River, New York. N.Y. Fish Game J. 28(1): 49–60.
- 1981b. Natural hybridization of *Oncorhynchus kisutch* and *O. tshawytscha* in a Lake Ontario tributary, with notes on meristic variation. *Copeia* 3: 720–721.
- KLINE, T. C. JR., J. J. GOERING, O. A. MATHISEN, P. H. POE, AND P. L. PARKER. 1990. Recycling of elements transported upstream by runs of Pacific salmon: I. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ evidence in Sashin Creek, southeastern Alaska. *Can. J. Fish. Aquat. Sci.* 47: 136–144.
- KROHKIN, E. M. 1967. Effect of size of escapement of sockeye salmon spawners on the phosphate content of a nursery lake. *Izvestiya Tikhookean. Nauchno-Issled. Inst. Rybn. Khoz. Okeanogr.* 57. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. 1186: 31–54)
- LEBRASSEUR, R. J., C. D. MCALLISTER, AND T. R. PARSONS. 1979. Addition of nutrients to a lake leads to greatly increased catch of salmon. *Environ. Conserv.* 6(3): 187–190.
- LOWE, R. L., S. W. GOLLADAY, AND J. R. WEBSTER. 1986. Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. *J. N. Am. Benthol. Soc.* 5(3): 221–229.
- MEYER, J. L., AND G. E. LIKENS. 1979. Transport and transformation of phosphorus in a forest stream ecosystem. *Ecology* 60(6): 1255–1269.
- MILLER, T. S., D. A. SHERWOOD, AND M. M. KREBS. 1989. Hydrogeology and water quality of the Tug Hill glacial aquifer in northern New York. USGS/WRI 88-4014.
- MINSHALL, G. W., E. HITCHCOCK, AND J. R. BARNES. 1991. Decomposition of rainbow trout (*Oncorhynchus mykiss*) carcasses in a forest stream ecosystem inhabited only by nonanadromous fish populations. *Can. J. Fish. Aquat. Sci.* 48: 191–195.
- MURPHY, J., AND J. P. RILEY. 1962. A modified single solution for the determination of phosphate in natural waters. *Anal. Chim. Acta* 9, 27, 31.
- NAIMAN, R. J., AND J. R. SEDELL. 1980. Relationships between metabolic parameters and stream order in Oregon. *Can. J. Fish. Aquat. Sci.* 37: 834–847.
- NEGULESCU, M., AND V. ROJANSKI. 1969. Recent research to determine reaeration coefficient. *Water Res.* 3(3): 189–202.
- ODUM, H. T. 1956. Primary production in flowing waters. *Limnol. Oceanogr.* 1: 102–117.
1983. Systems ecology. John Wiley and Sons, New York, NY. 644 p.
- PARSONS, J. W. 1973. History of salmon in the Great Lakes, 1850–1970. U.S. Fish Wildl. Serv. Tech. Pap. 68: 80 p.
- PETERSON, B. J., J. E. HOBBS, A. E. HERSHEY, M. A. LOCK, T. E. FORD, J. R. VESTAL, V. L. MCKINLEY, M. A. J. HULLAR, M. C. MILLER, R. M. VENTULLO, AND G. S. VOLK. 1985. Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. *Science (Wash., DC)* 229: 1383–1386.
- PRINGLE, C. M. 1987. Effects of water and substratum nutrient supplies on lotic periphyton growth: an integrated bioassay. *Can. J. Fish. Aquat. Sci.* 44: 619–629.
- RAND, P. S. 1990. The effects of salmon migrations on phosphorus dynamics and primary productivity in two Lake Ontario tributary streams. M.S. thesis, SUNY College of Environmental Science and Forestry, Syracuse, NY. 260 p.
- RICHEY, J. E., M. A. PERKINS, AND C. R. GOLDMAN. 1975. Effects of kokanee salmon (*Oncorhynchus nerka*) decomposition on the ecology of a subalpine stream. *J. Fish. Res. Board Can.* 32: 817–820.
- RIGLER, F. H. 1979. The export of phosphorus from Dartmoor catchments: a model to explain variations of phosphorus concentrations in streamwater. *J. Mar. Biol. Assoc. U.K.* 59: 659–687.
- STRICKLAND, J. D. H., AND T. R. PARSONS. 1968. A practical handbook of seawater analysis. *Bull. Fish. Res. Board Can.* 167: 319 p.
- WEBSTER, D. A. 1982. Early history of the atlantic salmon in New York. N.Y. Fish Game J. 29(1): 26–44.
- WISNIEWSKI, S. J. 1990. Secondary production of salmonids in tributaries of the Salmon River, New York. M.S. thesis, SUNY College of Environmental Science and Forestry, Syracuse, NY. 115 p.

Population Genetic Structure of the Armorhead, *Pseudopentaceros wheeleri*, in the North Pacific Ocean: Application of the Polymerase Chain Reaction to Fisheries Problems

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Armorhead (*Pseudopentaceros wheeleri*) occur in the subarctic, epipelagic habitats of the northern Pacific Ocean and are known to reproduce on seamounts in the central Pacific. Over the last few decades, overexploitation of seamount populations led to dramatic declines in abundances of reproductive populations. We undertook a study of the population genetics of armorhead to test whether distinct stocks exist in association with specific seamounts. We used the polymerase chain reaction (PCR) and a combination of DNA sequencing and restriction fragment length polymorphism (RFLP) analysis to analyze mtDNA variants for individuals collected from three localities: two seamounts and from the open ocean. We discovered that mtDNA haplotypes are not partitioned geographically, refuting the hypothesis that different seamounts harbor genetically distinct populations. Furthermore, genetic similarity of seamount and open-ocean fish supports the hypothesis that armorhead migrate between the central and northern Pacific Ocean for reproduction and feeding, respectively.

L'espèce *Pseudopentaceros wheeleri* vit dans les habitats subarctiques épipélagiques de la région septentrionale de l'océan Pacifique et on sait qu'elle se reproduit sur les monts sous-marins de la région centrale de cet océan. Au cours des dernières décennies, la surexploitation des populations des monts sous-marins a conduit à des déclin spectaculaires de l'abondance des populations reproductrices. Nous avons entrepris une étude de la génétique des populations de *P. wheeleri* afin d'examiner s'il existe des stocks distincts en association avec des monts sous-marins particuliers. Nous avons utilisé la réaction en chaîne de la polymérase (RCP) et une combinaison de séquençage d'ADN et d'analyse du polymorphisme de la longueur des fragments de restriction (PLFR) pour analyser les variants d'ADNmt de sujets prélevés en trois endroits : deux monts sous-marins et la haute mer. Nous avons découvert que les haplotypes d'ADNmt ne suivent pas une compartimentation géographique, ce qui réfute l'hypothèse selon laquelle des monts sous-marins différents seraient peuplés par des populations génétiquement distinctes. En outre, la similarité génétique des poissons des monts sous-marins et des poissons de haut mer corrobore l'hypothèse selon laquelle *P. wheeleri* migre entre la région centrale et la région septentrionale de l'océan Pacifique respectivement à des fins d'alimentation et de reproduction.

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The hypothesized life history of armorhead (*Pseudopentaceros wheeleri*) is extraordinary. Eggs and larvae are planktonic. Larvae rapidly develop and assume a nektonic habit. At this stage, juveniles move away from the seamounts and subadult fish are most often observed in the cold, nutrient-rich waters of the North Pacific (Boehlert and Sasaki 1988). After feeding for at least 1–3 yr, individuals are believed to migrate to the seamounts in the central Pacific Ocean, take up a demersal habit, spawn, and die (Humphreys et al. 1989).

Armorhead have been recorded from over a dozen seamounts in the central North Pacific Ocean (Borets 1979; Humphreys

and Tagami 1986). There are often differences in morphology among individual fish captured in the vicinity of a particular seamount as well as among fish captured at different seamounts. Such variation led Hardy (1983) to recognize two distinct species: *P. pectoralis* and *P. wheeleri*. Subsequent work by Humphreys and Tagami (1986) and Humphreys et al. (1989) showed that the morphological variation exhibited by armorhead is associated with the transition from a pelagic to demersal existence coupled with the onset of reproduction. Just after transition from the pelagic stage, individuals have high fat reserves, and ratios of body depth to fork length are usually greater than 0.25. Over time, individuals become leaner as fat reserves are used up, exhibiting body depth to fork length ratios

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of 0.20 and less. Regional differences in morphology among fish are thought to be due to differences in age at recruitment to the demersal stage.

The existence of a single species of armorhead in the North Pacific is supported by allozyme data that show no allele frequency differences between morphologically distinguishable fish from the Hancock Seamount in the Northern Hawaiian Ridge (NHR) (Humphreys et al. 1989). However, Humphreys et al. analyzed fish from only a single seamount, leaving open the possibility that fish at other seamounts, differing in latitude, oceanographic characteristics, size, proximity to other seamounts, and productivity (Uchida et al. 1986), are genetically different.

Elucidation of genetic relationships among the different seamount populations is warranted because armorheads were once (and may again be) commercially important. Furthermore, only the southernmost seamounts (Hancock Seamounts) lie within U.S. waters whereas the remainder are in international waters. If independent gene pools are associated with different physiogeographically distinct seamounts, then efforts to manage the exploitation of these fish can be implemented on a seamount-by-seamount basis. On the other hand, lack of differentiation among fish from different seamounts would suggest that there is a single panmictic population that is widely distributed throughout the North Pacific. In this case, exploitation of these fish anywhere in the North Pacific may impact the entire stock, and management tactics should be implemented accordingly.

We investigated the population genetics of *P. wheeleri* collected from the two seamounts separated by approximately

500 km and from the open ocean (Fig. 1) to determine if there was evidence for genetic differentiation among fish captured near different seamounts. We also determined haplotypes of fish captured in the open ocean and compared them with haplotypes of fish captured near seamounts to provide indirect evidence for migration between sites of reproduction in the central Pacific Ocean and the hypothesized feeding grounds in the North Pacific Ocean. Finally, we compared the haplotypes for morphologically different fishes to test whether there is a relationship between morphological and genetic differentiation and if there is genetic evidence for two species in the North Pacific Ocean. Our approach employed a combination of DNA sequence and restriction fragment length polymorphism (RFLP) analysis of polymerase chain reaction (PCR) amplified gene regions of mitochondrial DNA (mtDNA). These techniques for population genetic analysis of *P. wheeleri* can be applied to other species of interest.

Methods and Materials

Collection of Fish

Demersal adult fish were caught by pelagic longline from two seamounts, Koko and southeast Hancock in the central North Pacific (Fig. 1). Pelagic-phase juveniles were obtained from a region bounded by latitudes 41–44°N and longitudes 157–165°W (Fig. 1) by foreign drift-net vessels. For the fish caught at the seamounts, portions of the liver and oocytes (when present) were initially frozen in liquid nitrogen and then stored at –70°C until analyzed. For the pelagic fish, 1-g samples of



FIG. 1. Map of the Northwestern Hawaiian Islands – Southern Emperor Seamounts showing the location of collection sites for armorheads. Collections were obtained from Koko Seamount, Hancock Seamount, and for pelagic-phase fish from a region bounded by 41–44°N latitude and 157–165°W longitude.

TABLE 1. Primers used for PCR amplification. H and L refer to heavy or light strand, respectively. Numbers correspond to the location of the primer sequence in the human genome sequence (Anderson et al. 1981). Primer sequences are given 5' to 3'. Y = C and T. See Palumbi et al. (1991b) for more information.

Primer	Sequence
Cytochrome oxidase I	
COI-L6569	CCTGCAGGAGGAGGAGAYCC
COI-H7110	CCAGAGATTAGAGGGAATCAGTG
Hypervariable D-loop	
CB3-L15560	CATATTAAACCCGAATGATATTT
12SA-H1067	ATAATAGGGTATCTAATCCTAGTTT
Ribosomal genes	
12SA-L1067	AAACTGGGATTAGATACCCCACTAT
16SA-H2492	ATGTTTTTGATAAACAGGCG

muscle were placed in homogenization buffer (see below) and stored at 4°C.

Extraction of DNA

Approximately 1 g of tissue was homogenized in 2–3 mL of buffer (250 mM sucrose, 50 mM EDTA, 50 mM Tris, pH 7.5) using a Teflon-glass homogenizer and 1 mL of the homogenate was placed into a 1.5-mL Eppendorf tube. The homogenate was centrifuged for 2 min at 1000g at room temperature. The supernatant was transferred to a new tube and centrifuged at 8000g for 3 min. The resulting pellet was resuspended in 600 μ L of fresh buffer and lysed by the addition of sodium dodecyl sulfate (an ionic detergent) to a concentration of 1%. The lysate was extracted once with phenol, once with phenol–chloroform (1:1), and once with chloroform – isoamyl alcohol (24:1). The nucleic acids were precipitated with ethanol and 2.5 M ammonium acetate (pH 7.5), vacuum dried, resuspended in 50 μ L of 0.1 $\times$ TE, and stored at –20°C. When oocytes were present in individuals, a simpler and quicker method of DNA extraction was substituted. Like most species of teleosts, the oocytes of armorhead are small (<1 mm in diameter). Two to 10 oocytes were suspended in 50 μ L of buffer (50 mM KCl, 10 mM Tris, pH 8.3, 1 mM EDTA) and the cells lysed by the addition of NP40 (a nonionic detergent) to a concentration of 1%. The sample was boiled at 95°C for 5 min and sterile distilled water added to a final volume of 100 μ L.

Amplification of Mitochondrial Gene Regions

Specific gene regions of the mitochondrial genome were amplified using the PCR (Erich 1989; Palumbi et al. 1991b). Double-stranded DNA was amplified in 50 μ L reactions containing 50 mM KCl, 10 mM Tris, 1.5 mM MgCl₂, 0.01% gelatin, 0.01% Triton-X, 0.01% NP40, 1 μ M each of oligonucleotide primer, 200 μ M each of dNTP, 0.5 unit of Taq polymerase, and 1 μ L of the DNA sample. Thermal cycling parameters were as follows: denaturation at 94°C for 40 s, annealing at 55°C for 25 s, and elongation at 72°C for 1–2 min. For the purpose of sequencing, single-stranded templates were generated by adding 1 μ L of the double-stranded reaction to a 100- μ L reaction containing only a single primer and all of the amplification reagents. The same conditions for single-stranded amplification were used as in double-stranded amplifications. Primers were synthesized on a Biosystems PCR Mate Oligonucleotide Synthesizer.

We routinely amplified three distinct gene regions of the mitochondrial genome of *P. wheeleri*: a 450-bp region of the cytochrome oxidase I gene, a 1425-bp region comprising most

of the 12S rRNA gene and the 5' half of the 16S rRNA gene, and an approximately 2000-bp fragment containing the entire D-loop including flanking gene regions on both sides. Primer sequences are given in Table 1.

DNA Sequencing

Single-stranded templates were purified by centrifugal ultrafiltration through Centricon 30 tubes. DNA sequencing of the cytochrome oxidase fragment was performed using the Sequenase system (USBiochemicals). Fragments were separated through 6% acrylamide, 7 M urea buffer – gradient gels (Palumbi et al. 1991b) and the radioactive bands from the sequencing reaction were visualized by exposure to X-ray film for 24–48 h (Fig. 2).

Endonuclease Restriction Analysis

The ribosomal genes and D-loop region were amplified as described and 12 μ L of the amplified sample subjected to endonuclease digestion using the four-base recognition enzymes *Hae* III, *Mbo* I, and *Msp* I. Digestions were performed directly in the PCR buffer at 37°C for 4–6 h. The DNA fragments were separated in 1–1.5% agarose gels, stained with ethidium bromide, and the fragments made fluorescent by exposure to ultraviolet light and photographed (Fig. 3). The presence or absence of restriction sites was inferred from comparisons of fragment patterns (Palumbi et al. 1991a), and composite genotypes were assigned to each individual.

Genetic Relationship among Genotypes

The topology of relationships among genotypes was determined using the principle of parsimony (PAUP, Swofford 1989), treating the restriction site changes as Dollo character types. Restriction sites in the ribosomal and D-loop fragment were assigned weights of 5 and 1, respectively, reflecting the fact that ribosomal genes evolve considerably slower than the D-loop (Cann et al. 1987). Equal weights were also considered. We tested for differentiation among the seamount and open-ocean fish by standard contingency analysis and the G_{ST} test of Takahata and Palumbi (1985). Significance levels were determined using randomization tests (Roff and Bentzen 1989; Palumbi and Wilson 1990). In addition, average nucleotide diversity was estimated following Takahata and Palumbi (1985). The estimation of migration rate, Nm , was done using the method of Slatkin and Maddison (1989) based on the inferred topology of the relationships among distinct alleles.

Results

DNA Sequence Analysis

DNA sequences were determined for a 353-bp region of the cytochrome oxidase I gene for 10 individuals from each of the two seamounts (Fig. 2). Only a single C–T transition nucleotide substitution at a silent site (third “wobble” position of a codon) was found that distinguishes two mtDNA genotypes. This sequence variant was observed in four individuals: three from Koko and one from southeast Hancock Seamounts, respectively. None of the pelagic fish were sequenced. Based on all pairwise sequence comparisons for this gene region, the average (± 1 SD) nucleotide diversity of $0.10 \pm 0.14\%$. However, because most of the nucleotide positions (approximately two thirds) of the cytochrome oxidase I gene are invariable due to selective constraints on the protein, a more


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CTT GGC TAC ATA GGC ATA GTG TGA GCT ATA ATA GCA ATC GGC CTC CTA
T.. ..A ... ..T ..G ..C ... ..G ... T.. ..T ... T.. ...

GGG TTT ATT GTC TGG GCC CAC CAC ATG TTC ACA GTG GGA ATA GAC GTA
... ..C ..G ..A ..A ... ..T ..A ..T ... ..A ... ..T ...

GAC ACC CGT GCC TAC TTC ACG TCC GCC ACT ATA ATT ATC GCA ATT CCC
... ..A ..A ..A ..T ... ..C ... ..T ..C ... ..C ... ..T ..C ...

ACC GGT GTT AAA GTT TTC AGC TGA CTC GCC ACC CTC CAT GGG GGA AAC
... ..C ..C ... ..A ..T ... ..T ... ..A ... ..C ..A A.C ...T

ATC AAA TGA GAC ACA CCA CTT CTA TGA GCC CTA GGC TTT ATC TTC CTC
..G ... ..TCT G.T G.. G.G ..C ... ..T ... ..A ..C ... ..T ..T

TTT ACA GTA GGG GGC TTA ACA GGG ATT ATC CTT GCC AAY TCT TCT TTA
..C ..C ... ..T ... C.G ..T ..C ... G.A T.A ..A ..C ..A ..A C..

GAC ATT GTG CTC CAT GAC ACA TAC TAT GTA GTA GCC CAC TTC CAC TAC
... ..C ..A ..A ..C ... ..G ... ..C ..T ... ..T ... ..T ...

GTA CTA TCA ATA GGA GC
..C ... ..T ... ..T ...

```

FIG. 2. A 353-bp DNA sequence for a region of the cytochrome oxidase I gene from armorhead (above) aligned with the homologous region from human (below). The only variable nucleotide position detected from analysis of 20 individuals is a C-T transition mutation represented as a Y in the armorhead sequence and marked by an asterisk. The human cytochrome oxidase I sequence begins at position 6705 in the published sequence (Anderson et al. 1981).

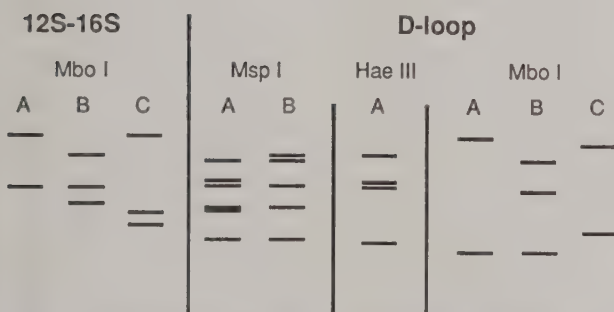


FIG. 3. Fragment pattern profiles for endonuclease digestions of the 12S-16S ribosomal gene regions and the D-loop.

informative estimate is the average percent sequence divergence at silent, third codon ("wobble") positions. Based on these data, this value is $0.30 \pm 0.42\%$.

Endonuclease Restriction Site Analysis

A 1425-bp fragment containing large portions of the 12S and 16S rRNA genes as well as the lysine transfer RNA was amplified for all individuals. Although this region is conserved in all metazoans examined to date, there are short variable regions that are useful for describing intraspecific relatedness. Digestion of this fragment with *Mbo* I detected three different fragment patterns inferred to be the result of polymorphisms at two restriction sites (Fig. 3). Digestion profiles for *Hae* III and

TABLE 2. Comparison of estimates of nucleotide diversity based on direct DNA sequence analysis and indirect restriction site analysis. *D* is the average percent nucleotide diversity. Length represents the approximate amount of the mtDNA genome (in base pairs) represented by the gene region. *N* is the average number of nucleotide differences between two individuals estimated as $(D \times \text{length})/100$. S = sequencing and R = restriction site analysis.

Gene region	<i>D</i>	Length	<i>N</i>	Method
Protein coding	0.10	12 000	12	S
Ribosomal	0.64	2 700	17.3	R
D-loop	1.87	1 000	18.7	R
Total		≈16 000	48	

Msp I revealed four and one restriction sites, respectively, that were monomorphic for 17 of the individuals analyzed by sequencing and thus were not used for the remainder of the samples. (Three individuals that were sequenced were omitted from restriction site analysis because PCR amplification and subsequent endonuclease digestions yielded uninterpretable results.) For the 17 individuals surveyed with three 4-bp recognition enzymes, nucleotide diversity in the ribosomal fragment was estimated as 0.64%.

We also developed primers that routinely amplify an approximately 2000-bp fragment containing the entire D-loop and adjacent flanking regions. We focused on this region because most intraspecific mtDNA nucleotide variation occurs in this region (Cann et al. 1987). The hypervariability of the D-loop is thought to be due in part to frequent length mutations and lack of constraint on sequence change (Saccone et al. 1987).

Initial screening of this region with *Hae* III, *Mbo* I, and *Msp* I for the same 17 individuals analyzed for ribosomal gene variation and cytochrome oxidase I sequences showed an average nucleotide diversity for this region of 1.87%, greater than 10 times the amount of variation detected by sequencing a small portion of the cytochrome oxidase I gene (Table 2).

Population Differences

For both ribosomal and D-loop regions, an additional 67 individuals were surveyed using restriction digestion analysis. A total of 10 distinct mtDNA types were detected. PAUP analysis yielded two most parsimonious trees depicting the relationships among the mitochondrial haplotypes. We used the strict consensus option in PAUP to construct a topology which summarizes the information contained in the two most parsimonious trees (Fig. 4).

The most common type (designated A) was identified in over 64% of the fish surveyed. The frequency of occurrence of the next most common type was only 9.2%. The remaining types were rare, with frequencies of occurrence of 5% or less. Based on the restriction site data for all individuals combined, the overall average percent sequence diversity in the ribosomal and D-loop regions was 1.2%.

No significant differences in allele frequency were detected among the three populations surveyed (chi-square = 14.4, $df = 16$, $p > 0.5$). G_{ST} analysis revealed that 6.7% of the genetic variation is distributed among groups. Bootstrap analysis indicates that this level of partitioning of genetic variation is not significantly different from random ($p > 0.25$). Using the approach of Slatkin and Maddison (1989), we estimated that, on average, greater than 10 mitochondrial haplotypes have been exchanged among the three populations (Koko, southeast Hancock, and pelagic phase) per generation ($Nm > 10$). (This result also holds for the topology generated from unweighted data.) Theory suggests that for Nm values greater than unity,

there is sufficient gene flow among populations to inhibit genetic differentiation by drift. Thus, the genetic data do not provide evidence for significant population structure of armorhead in the North Pacific.

The individual fish sampled from the two seamounts were identified as being either lean, intermediate, or fat types according to the ratio of fork length to body depth categories defined by Humphreys et al. (1989). A contingency test showed no significant genetic differentiation among the three morphological classes (chi-square = 18.3, $df = 20$, $p = 0.57$).

Discussion

Variability of mtDNA in Armorhead

The cytochrome oxidase I sequence data indicated a low level of intraspecific nucleotide variability in this species. Only a single variable position was observed in 7020-bp surveyed (20 individuals $\times$ 351-bp). To our knowledge, this is the lowest level of mtDNA nucleotide variability documented for a vertebrate species using these techniques. By contrast, there is appreciable levels of nuclear gene diversity (average heterozygosity = 0.077 (Humphreys et al. 1989)). If the low level of mtDNA diversity is a real phenomenon, then this discrepancy in estimates of genetic variation may indicate that populations of these fish underwent a transient bottleneck event, perhaps relatively recently (Wilson et al. 1985).

It is important to point out, however, that at this time we are unable to directly compare the estimate of nucleotide diversity in armorhead with other taxa because we sampled only a small section of a single gene whereas most other studies of mtDNA variability have employed RFLP analysis of whole mtDNAs (e.g. Graves et al. 1984; Avise et al. 1987). Although we surveyed 117 silent sites (the third "wobble" positions of codons), this amount of sequence is insufficient for reliable estimation of genetic divergence between mtDNAs (Martin

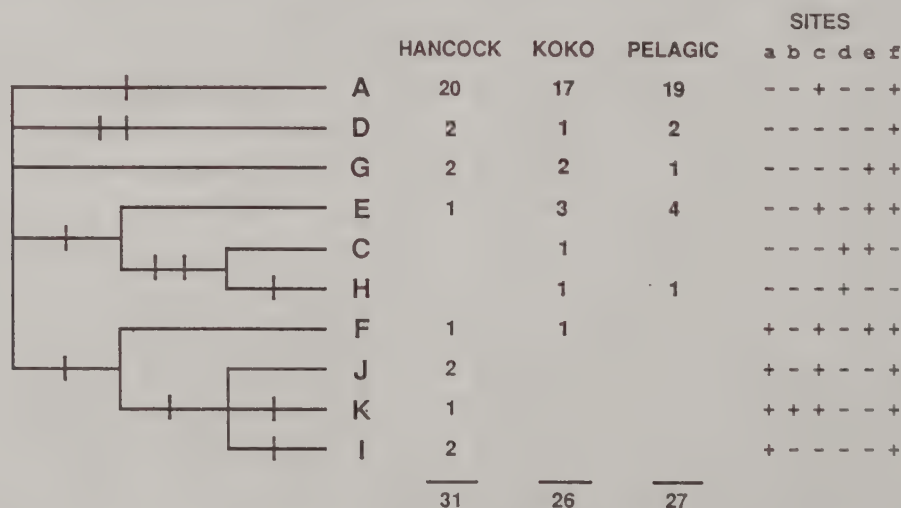


FIG. 4. Inferred topology of the relationships among the 10 mtDNA types identified by endonuclease restriction analysis. Individual tick marks represent site gains or losses. Sites are presented as gains (+) or losses (-) of restriction sites based on the fragment patterns in Fig. 3. Numbers of individuals possessing a particular mtDNA type are given. Site designations are as follows (see Fig. 3 for reference): a, 12S-16S *Mbo* I site gain from A to B; b, 12S-16S *Mbo* I site gain from A to C; c, D-loop *Msp* I site loss from A to B; d, D-loop *Mbo* I site gain from A to C; e, D-loop *Mbo* I site loss from B to A; f, D-loop *Mbo* I site loss from A to C. Consistency index, excluding uninformative sites, is 0.70.

et al. 1990). Thus, the low level of mtDNA sequence variability detected may be a real phenomenon or reflect the limited number of base pairs sampled; therefore, the transient bottleneck hypothesis is highly speculative.

Population Structure

The mtDNA data indicated lack of differentiation between fish collected at different seamounts and from the open ocean. These data provide support for the hypothesized life history and suggest that individuals born at the southernmost seamounts, for example, are equally as likely to reproduce at northern seamounts as at southern seamounts in the next generation. However, we detected few genetic markers that afforded limited resolution of within-species genealogy making it possible that further analysis of armorhead may reveal significant genetic differentiation among fish collected near different seamounts.

One or Two Species?

Based on analysis of the cytochrome oxidase I sequence data and restriction digestion profiles, there is no evidence for genetic differentiation between the three morphotypes of armorhead, identified as lean, intermediate, and fat using the ratio of body depth to mean fork length (Humphreys et al. 1989). In addition to analyses of morphology and allozyme variation, the lack of mtDNA differentiation between morphotypes provides convincing evidence for the existence of only a single species of North Pacific armorhead.

D-loop Amplification for Rapid Genetic Analysis of Fish Populations

DNA sequence analysis provides high-resolution data for population genetic studies because individual nucleotide substitutions can be identified. Unfortunately, DNA sequencing is more expensive and time consuming than indirect but lower resolution methods like restriction site analysis. We find that restriction site analysis of amplified D-loop fragments allowed detection of more nucleotide variation than direct sequence analysis of a 353-bp region of the cytochrome oxidase I gene (Table 2). Although DNA sequence data of D-loops provide superior resolution of genetic relationships among individuals (Meyer et al. 1990; di Rienzo and Wilson 1991), it is possible to distinguish mtDNA types using our technique of PCR amplification of the hypervariable D-loop and endonuclease restriction analysis. This method is rapid, does not require freezing tissue samples to -70°C , and does not involve radioactivity for visualization of DNA, enabling analysis of large numbers of individuals for only a fraction of what sequencing would cost. In addition, variant genotypes identified by restriction digestion can be sequenced directly from the amplification reaction (Palumbi et al. 1991b) in order to ascertain exact sequence divergence.

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References

- ANDERSON, S., A. T. BANKIER, B. G. BARRELL, M. H. L. DEBRUIJN, A. R. COULSON, J. DROUIN, I. C. EPERON, D. P. NIERLICH, B. A. ROE, F. SANGER, P. H. SCHEIER, A. J. H. SMITH, R. STADEN, AND I. G. YOUNG. 1981. Sequence and organization of the human mitochondrial genome. *Nature (Lond.)* 290: 457-465.
- AVISE, J. C., C. A. REEB, AND N. C. SAUNDERS. 1987. Geographic population structure and species differences in mitochondrial DNA of mouthbrooding marine catfishes (Ariidae) and demersal spawning toadfishes (Batrachoididae). *Evolution* 41: 991-1002.
- BOEHLERT, G. W., AND T. SASAKI. 1988. Pelagic biogeography of the armorhead, *Pseudopentaceros wheeleri*, and recruitment to isolated seamounts in the North Pacific Ocean. *Fish. Bull.* 86: 453-465.
- BORETS, L. A. 1979. The population structure of the boarfish, *Pentaceros richardsoni*, from the Emperor Seamounts and the Hawaiian Ridge. *J. Ichthyol.* 19: 15-20.
- CANN, R. L., M. STONEKING, AND A. C. WILSON. 1987. Mitochondrial DNA and human evolution. *Nature (Lond.)* 325: 31-36.
- DI RIENZO, A., AND A. C. WILSON. 1991. Branching pattern in the evolutionary tree for human mitochondrial DNA. *Proc. Natl. Acad. Sci. USA* 88: 1597-1601.
- ERLICH, H. A. 1989. PCR technology: principles and applications for DNA amplification. Stockton Press, New York, NY.
- GRAVES, J. E., S. D. FERRIS, AND A. E. DIZON. 1984. Close genetic similarity of Atlantic and Pacific skipjack tuna (*Katsuwonus pelamis*) demonstrated with restriction endonuclease analysis of mitochondrial DNA. *Mar. Biol.* 79: 315-319.
- HARDY, G. S. 1983. A revision of the fishes of the family Pentacerotidae (Perciformes). *N.Z. J. Zool.* 10: 177-220.
- HUMPHREYS, R. L. JR., AND D. T. TAGAMI. 1986. Review and current status of research on the biology and ecology of the genus *Pseudopentaceros*, p. 55-62. In R. N. Uchida, S. Hayasi, and G. W. Boehlert [ed.] *Environment and resources of seamounts in the North Pacific*. U.S. Dep. Commer. NOAA Tech. Rep. NMFS 43.
- HUMPHREYS, R. L., G. WINANS, AND D. T. TAGAMI. 1989. Synonymy and proposed life history of the North Pacific pelagic armorhead, *Pseudopentaceros wheeleri* Hardy (Pisces: Pentacerotidae). *Copeia* 1989: 142-153.
- MARTIN, A. P., B. D. KESSING, AND S. R. PALUMBI. 1990. Accuracy of estimating genetic distance between species from short sequences of mitochondrial DNA. *Mol. Biol. Evol.* 7: 485-488.
- MEYER, A., T. D. KOCHER, P. BASASIBWAKI, AND A. C. WILSON. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature (Lond.)* 347: 550-553.
- PALUMBI, S. R., A. P. MARTIN, B. D. KESSING, AND W. O. McMILLAN. 1991a. Detecting population structure using mitochondrial DNA, p. 271-278. In A. R. Hoelzel [ed.] *Cetacean genetics*. International Whaling Commission Publ. No. 13, Cambridge, U.K.
- PALUMBI, S. R., A. P. MARTIN, W. O. McMILLAN, S. ROMANO, L. STICE, AND G. GRABOWSKY. 1991b. The simple fool's guide to PCR. 2nd ed. University of Hawaii, Honolulu, HI.
- PALUMBI, S. R., AND A. C. WILSON. 1990. Mitochondrial DNA diversity in the sea urchins *Strongylocentrotus purpuratus* and *S. droebachiensis*. *Evolution* 44: 403-415.
- ROFF, D. A., AND P. BENTZEN. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Mol. Biol. Evol.* 6: 539-545.
- SACCONE, C., M. ATTIMONELLI, AND E. SBISA. 1987. Structural elements highly preserved during the evolution of the D-loop containing region in vertebrate mitochondrial DNA. *J. Mol. Evol.* 26: 205-211.
- SLATKIN, M., AND W. P. MADDISON. 1989. A cladistic measure of gene flow inferred from the phylogenies of alleles. *Genetics* 123: 603-613.
- SWOFFORD, D. 1989. Phylogenetic analysis using parsimony (PAUP) v. 3.0. Illinois Natural History Survey, Champaign, IL.
- TAKAHATA, N., AND S. R. PALUMBI. 1985. Extranuclear differentiation and gene flow in the finite island model. *Genetics* 109: 441-457.
- UCHIDA, R. N., S. HAYASI, AND G. W. BOEHLERT [ED.] 1986. Environment and resources of seamounts in the North Pacific. U.S. Dep. Commer. NOAA Tech. Rep. NMFS 43.
- WILSON, A. C., R. L. CANN, S. M. CARR, M. GEORGE, U. B. GYLLENSTEIN, K. M. HELM-BYCHOWSKI, R. G. HIGUCHI, S. R. PALUMBI, E. M. PRAGER, R. D. SAGE, AND M. STONEKING. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol. J. Linn. Soc.* 26: 375-400.

Acid and Aluminum Effects on Sodium Homeostasis and Survival of Acid-Sensitive and Acid-Tolerant Cladocera

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Acid-sensitive (*Daphnia galeata mendotae*) and acid-tolerant (*Bosmina longirostris*) cladocerans were exposed to 0, 100, or 200 $\mu\text{g Al/L}$ at pH 4.5, 6.0, or 7.5. Whole-body Na content and survivals were determined after 12- and 24-h exposures to the nine treatments. *Daphnia galeata mendotae* experienced significant decreases in body Na content and survival at pH 4.5. Aluminum effects were pH dependent. At pH 7.5 and 6.0, Na content and survival declined with increasing Al; at pH 4.5, the highest Al concentration enhanced Na content and prolonged survival. *Bosmina longirostris* Na content and survival were only slightly reduced at pH 4.5, and there were no significant Al or pH $\times$ Al interaction effects. The results support the view that (1) the extinction of *D. galeata mendotae* and the relative increase of *B. longirostris* during lake acidification are largely due to differential impacts of acid stress on osmoregulation and (2) Al toxicity might also be a factor responsible for *D. galeata mendotae* population declines, which are most pronounced near pH 6.0.

Des cladocères sensibles à l'acide (*Daphnia galeata mendotae*) et tolérants à l'acide (*Bosmina longirostris*) ont été exposés à des concentrations d'Al de 0, 100 ou 200 $\mu\text{g/L}$, à un pH de 4,5, 6,0 ou 7,5. La teneur en Na de l'organisme et la survie ont été déterminées après des expositions de 12 et de 24 h aux neuf traitements. Chez *D. galeata mendotae*, on a observé une diminution significative de la teneur en Na de l'organisme et de la survie à un pH de 4,5. Les effets d'Al étaient dépendants du pH. Lorsque le pH est égal à 7,5 et à 6,0, la teneur en Na et la survie diminuaient lorsque la concentration d'Al augmentait; avec un pH de 4,5, la concentration d'Al la plus élevée augmentait la teneur en Na et prolongeait la survie. Chez *B. longirostris*, la teneur en Na et la survie n'étaient que légèrement réduites à un pH de 4,5, et on n'a observé aucun effet significatif de l'Al ou de l'interaction pH et Al. Les résultats corroborent l'hypothèse suivante: (1) l'extinction de *D. galeata mendotae* et l'augmentation relative de *B. longirostris* durant l'acidification des lacs sont largement attribuables aux impacts différentiels du stress acide sur la régulation osmotique et (2) la toxicité de l'Al pourrait également jouer un rôle dans le déclin des populations de *D. galeata mendotae*, lequel atteint son maximum à un pH proche de 6,0.

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Zooplankton community structure is greatly affected by lake acidification. The total number of species is reduced (Confer et al. 1983; Siegfried et al. 1987; Havens 1991), community complexity declines (Havens 1991), and there are major changes in dominance (Confer et al. 1983; Pinel-Alloul et al. 1990; Tessier and Horwitz 1990). A conspicuous trend is the loss of large daphnids with declining pH (Carter et al. 1986; Malley and Chang 1986; Keller et al. 1990) and the increase in relative biomass of rotifers and small-bodied crustaceans like *Bosmina longirostris* and *Leptodiptomus minutus* (Sprules 1975; Yan and Strus 1980; Yan and Geiling 1985). Parameters like grazing rate, nutrient regeneration rate, and susceptibility to predation are related to zooplankton body size (Brooks and Dodson 1965; Hall et al. 1976; Chow-Fraser and Knoechel 1985; Henry 1985). Hence, the loss of large daphnids could affect overall food web function. An important question then is "why are some species eliminated from the zooplankton during acidification while others survive?" The answer is complex because both ecological and physiological effects may contribute to the loss of zooplankton species from acid-stressed communities (Nilssen et al. 1984). Furthermore, physiological changes might result from H^+ stress, or from other chemical stressors that increase in concentration during acidification, in particular aluminum. Aluminum typically increases (except in seepage lakes) from below 50 to over 200 $\mu\text{g/L}$ as pH falls from

above 6.5 to below 5 (Linthurst et al. 1986; Neary et al. 1990), and Al has been shown to be toxic to organisms at all trophic levels (e.g. Nalewajko and Paul 1985; Havens and Heath 1989; McDonald et al. 1989).

With regard to acid stress, Potts and Fryer (1979) suggested that H^+ ions interfere with osmoregulation in acid-sensitive zooplankton but not in acid-tolerant ones. They found that *Daphnia magna* (acid sensitive) experienced a net loss of Na^+ when exposed to low-pH water, while *Acantholeberis curvirostris* (acid tolerant) did not. Havas and Likens (1985a) obtained similar results for *D. magna*, as did Havas et al. (1984) with *D. magna* and *D. middendorffiana*. Both showed that Na^+ losses were followed by mortality. Havas and Likens also studied Al effects; they found that exposure to a high Al level (1000 $\mu\text{g/L}$) further reduced the Na^+ content and survival of *D. magna* exposed to pH 6.5 or 5.0 soft water. These results may provide an answer to the question asked above. However, since the studies involved either (1) littoral taxa (*A. curvirostris*), (2) limnetic taxa not common to northeastern North America (*D. magna* and *D. middendorffiana*), or (3) Al levels in excess of those found in precipitation-acidified lakes, it is uncertain whether they can be generalized to the limnetic zooplankton of the acid-impacted northeast.

My objective was to quantify the effects of acid and Al on osmoregulation and survival of *Daphnia galeata mendotae* and

B. longirostris. The approach was to expose test animals to various levels of pH and Al and to determine the effects on short-term (12 and 24 h) survival and Na content. Acid and Al levels were chosen to approximate those seen during lake acidification (pH 4.5–7.5, 0–200 $\mu\text{g Al/L}$), and the test species were chosen because of their numerical dominance in the plankton of northeastern North America.

Materials and Methods

Bioassays were performed on six dates during summer 1991, using test animals collected from the pelagic zones of East Twin Lake and Stewart Lake, Ohio, USA. These small glacial kettle lakes are alkaline (pH 7.9–8.7, total alkalinity 90–110 mg CaCO_3/L , total Al < 10 $\mu\text{g/L}$) and mesotrophic. East Twin was the study site for previous research dealing with acid and Al effects on the plankton (Havens and Heath 1989, 1990, 1991).

Toxicity tests were done on triplicate dates for each species, using a simulated soft water medium (Havas 1985) composed of deionized water plus 2.45 mg Ca/L, 0.52 mg Mg/L, 1.76 mg Na/L, 0.49 mg K/L, 2.07 mg Cl/L, and 5.3 mg SO_4/L . Soft water is optimal for bioassays of this type because the high Ca levels typical of hard-water lakes (like East Twin and Stewart) ameliorate H^+ and Al toxicity (Pagenkopf 1983; Havas et al. 1984) and make difficult the extrapolation of results to populations in acid-sensitive soft waters.

Aliquots (500 mL) of the medium were distributed into nine HCl-rinsed pyrex flasks. Three were spiked with 200 $\mu\text{g Al/L}$ as $\text{Al}_2(\text{SO}_4)_3$, three were spiked with 100 $\mu\text{g Al/L}$, and three were untreated controls. Trace metal grade H_2SO_4 and NaOH were used to adjust one flask at each Al level to pH 7.5, 6.0, and 4.5. The pH adjustments were done after the Al additions, and they resulted in nine pH/Al combinations. The Al levels were selected to cover the range of extractable Al concentrations reported for acid-sensitive soft lakes in the northeastern United States (Linthurst et al. 1986). Aliquots (50 mL) of each solution were pipetted into 27 HCl-rinsed plastic titration vessels (three per treatment) and the bioassay started shortly thereafter. Aluminum speciation was not measured in bioassays. However, equilibrium calculations based on pH, total Al, and the concentrations of ions present in the media suggest that at pH 4.5, most of the Al was ionic Al^{3+} , AlOH^{2+} , and AlSO_4^+ , while at pH 6.0 and 7.5, nearly all of the Al was solid $\text{Al}(\text{OH})_3$.

Zooplankton were collected with vertical hauls of a 300- μm plankton net in the lakes, and stored in bottles of unfiltered water until they were introduced into the bioassay vessels (1–2 h postsampling). Sample aliquots were placed into tissue culture plates under a dissecting microscope, and a Pasteur pipette was used to transfer groups of 10 animals into each of the 27 vessels. Following this procedure, which took roughly 1 h, the pH of each vessel was measured and readjusted if necessary. The vessels were placed on a wire rack and incubated at room temperature (19–22°C) for 24 h. It is noteworthy that the animals were not acclimated to the soft-water medium prior to the bioassays.

At 6, 12, and 24 h, pH was measured using an Orion model 520 meter with a Triode combination pH/ATC electrode. The pH changed less than 0.2 unit for all treatments in the six experiments. At 12 and 24 h, the animals in one vessel for each treatment were removed, in order to determine their survival and Na content (Na content was also determined at time 0). The contents of each vessel were rinsed into a plastic ring with a 200- μm -mesh base, and the retained animals were observed

at 30 $\times$ magnification. Animals lacking internal and external movement were classified as dead. In a separate experiment, Na content of unexposed animals and of animals exposed for 6 h to 200 $\mu\text{g Al/L}$ at pH 4.5 was determined. The animals were classified as live, dying, or dead prior to the analysis.

The groups of test animals were rinsed with deionized water, transferred with forceps into 0.2-mL plastic vials, and dried at 60°C for 24 h. Sodium contents were determined by neutron activation analysis (NAA) at The Ohio State University Reactor Laboratory. Vials were irradiated at 10^{12} neutrons $\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ for 2 h, in order to excite Na atoms to Na^{24} , a radioisotope that emits Ge photons at 1368 keV. Roughly 15 min after irradiation, the groups of animals were removed from their vials, weighed to ± 1 μg with an ultramicrobalance, and transferred to nonirradiated vials. Activities (microcuries per microgram, 1 $\mu\text{Ci} = 37$ kBq) were determined from 100- to 300-s counts on a high-purity Ge detector and were converted to concentration units (micrograms Na per gram dry weight) using a standard curve developed from U.S.N.B.S. standards irradiated and weighed along with the unknowns.

Two-way analysis of variance was used to test for pH, Al, and pH $\times$ Al interaction effects on survival and Na content of the test animals at 12 and 24 h. The term “significant” is used here to indicate $p < 0.05$. The data analyses were done using SAS Stat Version 5 (SAS Institute, 1985).

Results

Daphnia galeata mendotae

The bodily Na content of *D. galeata mendotae* (Fig. 1a) was roughly 8000 $\mu\text{g/g}$ dry weight at time 0 and did not differ significantly among the treatments. At 12 h (Fig. 1b), there was a significant pH effect on Na content; concentrations were reduced below 6000 $\mu\text{g/g}$ at pH 6.0 and below 2000 $\mu\text{g/g}$ at pH 4.5. The data also suggest a pH $\times$ Al interaction effect on Na content. At pH 7.5 and especially at pH 6.0, Na content was reduced in the high-Al treatment; at pH 4.5, Na content was enhanced in that same treatment. However, this interaction effect was not statistically significant. At 24 h (Fig. 1c), nearly identical results were obtained, except that Na content in the pH 4.5 treatments had further declined.

Trends in survival of *D. galeata mendotae* in the treatments were very similar to those for Na content. At 12 h, there was a significant pH effect on survival (Fig. 1d). Survival was slightly reduced at pH 6.0, and greatly reduced at pH 4.5, especially in the 0 and 100 $\mu\text{g Al/L}$ treatments. There was also a significant pH $\times$ Al interaction effect at 12 h. Survivals declined with increasing Al level at pH 7.5 and 6.0, while survival was enhanced in the high-Al treatment at pH 4.5. The 24-h results (Fig. 1e) were nearly identical, except that survival in the pH 4.5 treatments was further reduced.

Overall, there was a significant positive relationship between survival and bodily Na content in *D. galeata mendotae* (Fig. 2a). It is important to note that survival is presented as the dependent variable. This reflects the fact that when Na content was measured in live, dying, and dead animals after 6 h of exposure to the pH 4.5/high-Al treatment (Fig. 2b), nearly all of the Na loss preceded death.

Bosmina longirostris

The bodily Na content of *B. longirostris* was roughly 5000 $\mu\text{g/g}$ dry weight at time 0 (Fig. 3a), considerably lower than that measured for *D. galeata mendotae*. There were no

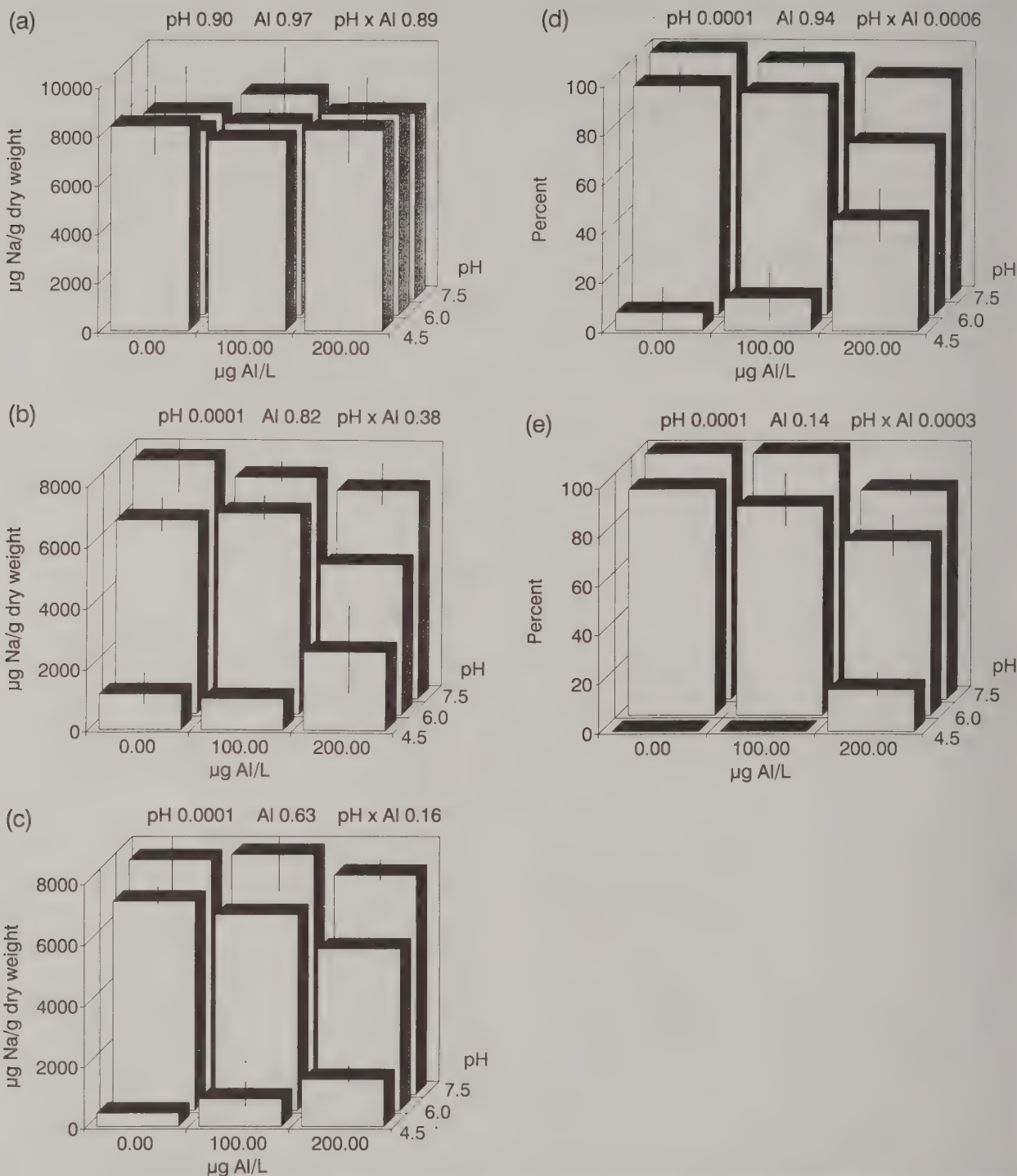


FIG. 1. Bodily Na content and survival of *D. galeata mendotae* in the nine bioassay treatments. Probability values from ANOVA for pH, Al, and pH $\times$ Al interaction effects are given above each column. Vertical bars are ± 1 SE. (a) Na content at time 0; (b) Na content after 12 h of exposure; (c) Na content after 24 h of exposure; (d) survival after 12 h of exposure; (e) survival after 24 h of exposure.

significant differences in initial Na levels among the treatments. At 12 h, Na content remained high (Fig. 3b), with the exception of slight declines at pH 4.5. There were no significant pH, Al, or pH $\times$ Al interaction effects. At 24 h, a significant pH effect on Na content was observed (Fig. 3c); levels

were reduced by roughly 20% in the pH 4.5 treatment. There were no significant Al or pH $\times$ Al interaction effects.

Trends in *B. longirostris* survival closely followed those for Na content. At 12 and 24 h (Fig. 3d, 3e), there were significant pH effects; survivals were reduced by roughly 10% at 12 h and

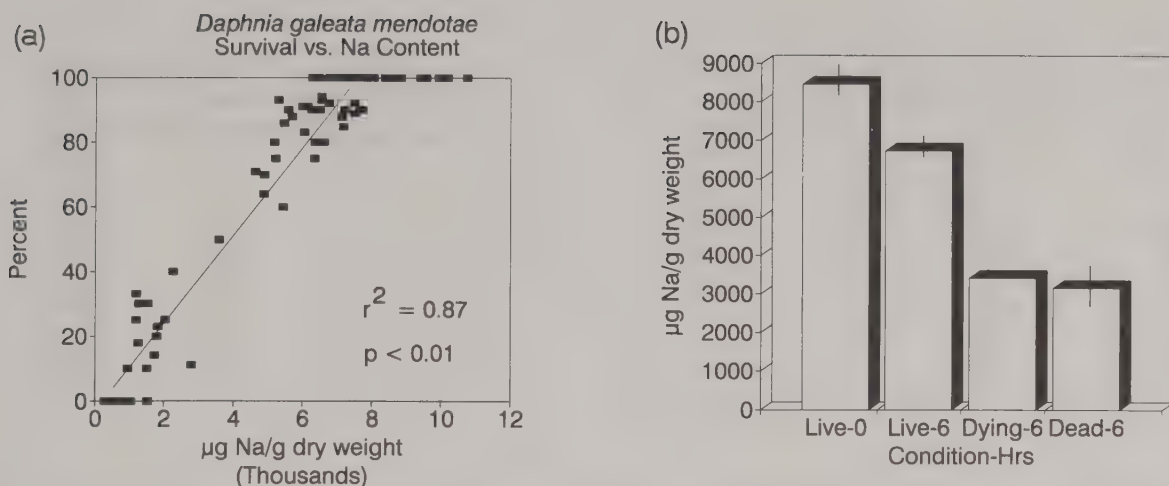


FIG. 2. Survival and Na content in *D. galeata mendotae*. (a) Percent survival versus Na content during the bioassay experiments; raw data points from the treatments are plotted; (b) Na content of live unexposed animals (Live-0) and of live (Live-6), dying (Dying-6) and dead (Dead-6) animals after 6 h of exposure to pH 4.5 and 200 µgAl/L.

20% at 24 h in the pH 4.5 treatment. There were no significant Al or pH $\times$ Al interaction effects on survival.

Survival and Na content in *B. longirostris* were not significantly correlated (Fig. 4). This reflects the high variability in Na content at high survival values and the fact that only slight declines occurred in the parameters during the experiments.

Discussion

Daphnia galeata mendotae is an important zooplankton species in northeastern North America. Keller et al. (1990) found that it occurred in 44–92% of pH >6.0 lakes in three Ontario data sets. N. D. Yan (pers. comm.) found it in 35% of lakes in central Ontario, and Tessier and Horwitz (1990) noted that *D. galeata mendotae* was a major contributor to the “large bodied zooplankton fauna” of alkaline lakes in the northeastern United States. Survey studies and laboratory toxicity tests have demonstrated that *D. galeata mendotae* is very sensitive to increased acidity. Keller et al. (1990) found that its biomass declined greatly in lakes of pH <6.0 in Ontario, and that it was absent from nearly all lakes of pH <5.5. The same authors also reported an LC₅₀ pH value of 5.85, in close agreement with the survey results. In the present study, the survival of *D. galeata mendotae* was somewhat reduced at pH 6.0, and all animals died within 24 h at pH 4.5. The decline in Na content of the animals suggests a mechanism of acid toxicity: the disruption of normal osmoregulation by elevated H⁺ concentrations. The same conclusion was reached by Potts and Fryer (1979) and Havas and Likens (1985a) for *D. magna*. In the former study, the animals experienced a decrease in Na influx and an increase in Na efflux at low pH. The implied net effect (not measured) was a reduction in whole-body Na content. Havas and Likens obtained the same results for influx and efflux rates and, upon measuring whole-body Na, did observe a significant decline in *D. magna* exposed to low-pH water. Havas (1985) noted that when animals lost large quantities of Na (and presumably other physiologically important ions), they were no longer able to coordinate their appendages. Hence, in nature, they would be unable to gather food, evade predators, or maintain themselves in the water column. I also observed that prior to death, animals experienced a great loss of bodily Na, and at

that point, they could not swim, but lay nearly motionless at the bottom of the bioassay vessels.

In contrast with these findings, exposure to low pH had only minimal effects on the survival and Na content of *B. longirostris*. This is consistent with the fact that this species is a common dominant in precipitation-acidified lakes. For example, Sutherland (1989) found the species in 23 of 28 Adirondack Mountain lakes having a pH below 5.0, Yan and Strus (1980) found it in 99% of samples taken from acidic Clearwater Lake, Ontario, and Havens and DeCosta (1985) found that it was the only cladoceran present in acidic Cheat Lake, West Virginia.

The consequences of obtaining test animals from hard-water lakes are important. Do such animals respond to acid stress in the same manner as their soft-water counterparts? For the two species used here, the answer is likely “yes”. In a related project (K. E. Havens, N. D. Yan, and W. Keller, unpubl.), laboratory LC₅₀ pH values were determined for *D. galeata mendotae* and *B. longirostris* taken from East Twin Lake. For *D. galeata mendotae*, the LC₅₀ value (pH 5.72 $\pm$ 0.09) was nearly identical to that (pH 5.85 $\pm$ 0.15) determined by Keller et al. (1990) for animals taken from a soft-water Ontario lake. For both *D. galeata mendotae* and *B. longirostris*, the toxicity tests results were also in close agreement with their distribution across pH gradients in Ontario and the Adirondack Mountains of New York. Mackie (1988) also noted that hard waters are a suitable site for collecting test animals for acid and metal toxicity tests because they preclude the possibility that populations have become adapted to the stressors, i.e. that they might consist of tolerant ecotypes. Another concern associated with the experimental protocol used here is that transfer from high- to low-Ca medium might have caused osmoregulatory stress and aggravated the effects of acid exposure. However, the high survivals and Na concentrations recorded in the pH 7.5, and 6.0, low-Al treatments indicate that this did not occur. Hence, the results suggest that differential effects of low pH on osmoregulation could be responsible for the shift from large daphnids like *D. galeata mendotae* to small species like *B. longirostris* during lake acidification. This is the same explanation given by Potts and Fryer (1979) for the absence of *D. magna* from

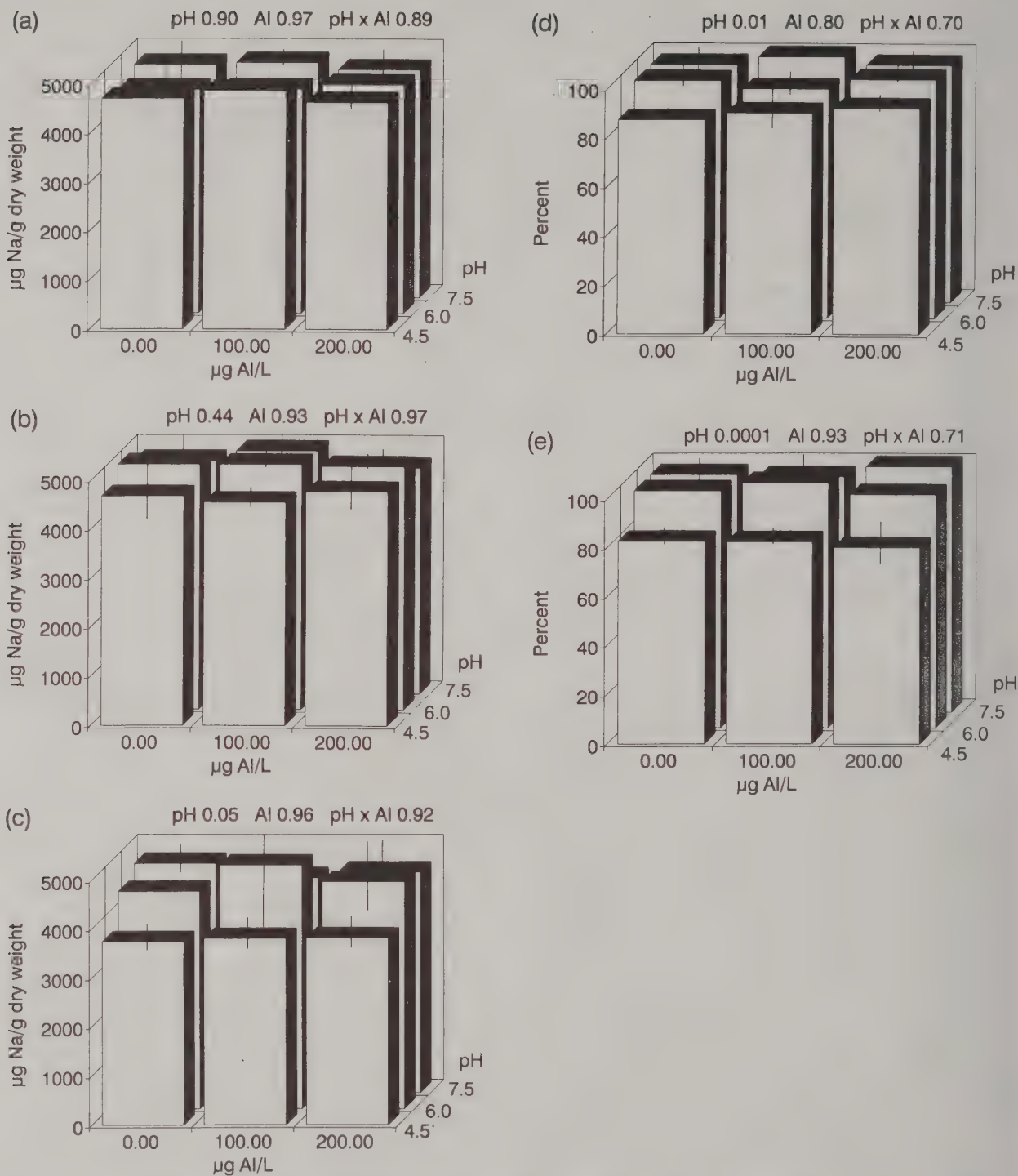


FIG. 3. Bodily Na content and survival of *B. longirostris* in the nine bioassay treatments. Symbols are as defined in Fig. 1. (a) Na content at time 0; (b) Na content after 12 h of exposure; (c) Na content after 24 h of exposure; (d) survival after 12 h of exposure; (e) survival after 24 h of exposure.

acidic waters in northern England and the increase in relative abundance of *A. curvirostris* in the littoral zone of those same lakes. Further research should be done to determine whether a similar mechanism can explain the shifts in dominance which

occur in the copepod and rotiferan zooplankton.

The effects of Al on the test animals were more complex, but again were consistent with the findings of previous studies. For *D. galeata mendotae*, exposure to the high Al level resulted

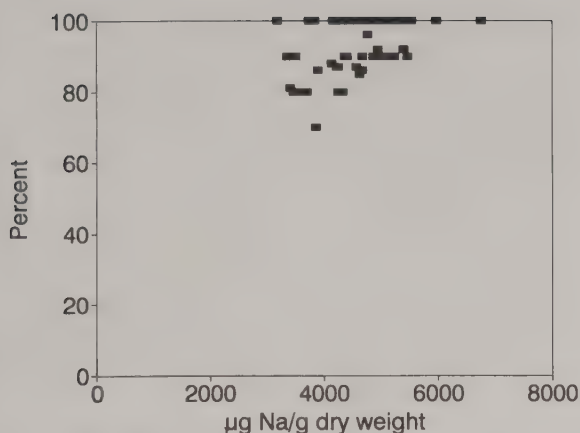


FIG. 4. Percent survival versus bodily Na content of *B. longirostris* during the bioassay experiments.

in reduced survival and Na content at pH 7.5 and especially at pH 6.0, but enhanced survival and Na content at pH 4.5. Similarly, Havas and Likens (1985a) found that at pH 6.5, (1000 µg/L) decreased Na influx in *D. magna* by 46% and increased Na efflux by 25%. This resulted in a net loss of Na and increased morbidity. At pH 4.5, Al did not affect Na influx, but decreased Na efflux by 31%. This reduced the net Na losses and enhanced the animal's survival. The pH × Al interaction effects on survival and Na content observed here and by Havas and Likens were likely a result of the pH-dependent speciation of Al and the differential toxicity of various forms of Al. In the pH 4.5 treatment, soluble ionic forms likely predominated. Havas and Likens suggested that at low pH, Al ions may act like Ca, reducing the permeability of membranes and protecting animals from ion losses. They also proposed a mechanism for the apparently greater toxicity of particulate $\text{Al}(\text{OH})_3$, the dominant species at higher pH. They suggested that a difference in pH (presumably lower) near the surface of target tissues might convert particulate Al to toxic ionic species like $\text{Al}(\text{OH})_2^+$. Filter-feeding zooplankton like *D. galeata mendotae* might be especially affected because their filtering activities would tend to concentrate particulates near the body surface.

The response of the acid-tolerant *B. longirostris* to Al exposure was consistent with the results of Havas and Likens (1985b), who found that Al (at 320 and 1000 µg·L⁻¹) had little effect on the survival of *Holopedium gibberum* and *Chaoborus punctipennis*, species common to the plankton of acid-stressed lakes. Havas and Hutchinson (1983) also found that *Chironomus riparius* from an acid pond did not experience a decline in bodily Na or Cl when exposed to high Al (667 000 µg/L). Havens and DeCosta (1987) suggested that acid-tolerant zooplankton are invariably Al tolerant. Some common mechanism of H⁺ and Al exclusion from sensitive target tissues may be involved. Indeed, Havens (1990) used hematoxylin staining and showed that upon exposure to µg Al/L, *D. galeata mendotae* experienced a great concentration of Al on the maxillary glands, while *B. longirostris* showed no evidence of Al on that osmoregulatory structure.

Because the detrimental effects of Al exposure on *D. galeata mendotae* were most pronounced at pH 6.0, where the species also seems to experience the greatest declines during acidification (Keller et al. 1990), one might conclude that Al toxicity is responsible in part for that decline. An important question, however, is whether the Al levels shown to be toxic here

actually occur in lakes at that pH. In fact they do. Neary et al. (1990) determined Al concentrations for 777 Ontario lakes. At pH 6, levels ranged from 10 to over 300 µg/L. While most lakes had below 100 µg/L, several had levels near 200. During spring snowmelt or other periods of rapid water influx, considerable increases in Al concentration occur. Hence, the Al levels used here were realistic.

In conclusion, the results of this study indicated that (1) the extinction of *D. galeata mendotae* and the increase of *B. longirostris* in many precipitation-acidified lakes could be largely due to differential impacts of acid stress on the species' osmoregulation, and consequently their survival, and (2) Al toxicity might also be a factor in the extinction of *D. galeata mendotae*.

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References

- BROOKS, J. L., AND S. I. DODSON. 1965. Predation, body size, and composition of plankton. *Science* (Wash., DC) 150: 28–35.
- CARTER, J. H. C., W. D. TAYLOR, R. CHENGALATH, AND D. A. SCRUTON. 1986. Limnetic zooplankton assemblages in Atlantic Canada with special reference to acidification. *Can. J. Fish. Aquat. Sci.* 43: 444–456.
- CHOW-FRASER, P., AND R. KNOEHEL. 1985. Factors regulating in situ filtering rates of Cladocera. *Can. J. Fish. Aquat. Sci.* 42: 567–576.
- CONFER, J. L., T. KAARET, AND G. E. LIKENS. 1983. Zooplankton diversity and biomass in recently acidified lakes. *Can. J. Fish. Aquat. Sci.* 40: 36–42.
- HALL, D. J., S. T. THRELKELD, C. W. BURNS, AND P. H. CROWLEY. 1976. The size-efficiency hypothesis and the size structure of zooplankton communities. *Annu. Rev. Ecol. Syst.* 7: 177–201.
- HAVAS, M. 1985. Aluminum accumulation and toxicity to *Daphnia magna* in soft water at low pH. *Can. J. Fish. Aquat. Sci.* 42: 1741–1748.
- HAVAS, M., AND T. C. HUTCHINSON. 1983. Effect of low pH on the chemical composition of aquatic invertebrates from tundra ponds at the Smoking Hills, N.W.T., Canada. *Can. J. Zool.* 61: 241–249.
- HAVAS, M., T. C. HUTCHINSON, AND G. E. LIKENS. 1984. The effects of low pH on sodium regulation in two species of *Daphnia*. *Can. J. Zool.* 62: 1965–1970.
- HAVAS, M., AND G. E. LIKENS. 1985a. Changes in ²²Na influx and outflux in *Daphnia magna* (Straus) as a function of elevated Al concentrations in soft water at low pH. *Proc. Natl. Acad. Sci. USA* 82: 7345–7349.
- 1985b. Toxicity of aluminum and hydrogen ions to *Daphnia catawba*, *Holopedium gibberum*, *Chaoborus punctipennis*, and *Chironomus anthracinus* from Mirror Lake, New Hampshire. *Can. J. Zool.* 63: 1114–1119.
- HAVENS, K. E. 1990. Aluminum binding to ion exchange sites in acid-sensitive and acid-tolerant cladocerans. *Environ. Pollut.* 64: 133–141.
- 1991. Crustacean zooplankton food web structure in lakes of varying acidity. *Can. J. Fish. Aquat. Sci.* 48: 1846–1852.
- HAVENS, K. E., AND J. DE COSTA. 1985. An analysis of selective herbivory in an acid lake and its importance in controlling phytoplankton community structure. *J. Plankton Res.* 7: 207–222.
- 1987. The role of aluminum contamination in determining phytoplankton and zooplankton responses to acidification. *Water Air Soil Pollut.* 33: 277–293.
- HAVENS, K. E., AND R. T. HEATH. 1989. Acid and aluminum effects on freshwater zooplankton: an *in situ* mesocosm experiment. *Environ. Pollut.* 62: 195–211.
- 1990. Phytoplankton succession during acidification with and without increasing aluminum levels. *Environ. Pollut.* 68: 129–145.
- 1991. Increased transparency due to changes in the algal size spectrum during experimental acidification in mesocosms. *J. Plankton Res.* 13: 1163–1176.
- HENRY, R. L. 1985. The impact of zooplankton size structure on phosphorus cycling in field enclosures. *Hydrobiologia* 120: 3–9.

- KELLER, W., N. D. YAN, K. E. HOLTZE, AND J. R. PITBLADO. 1990. Inferred effects of lake acidification on *Daphnia galeata mendotae*. *Environ. Sci. Technol.* 24: 1259–1260.
- LINTHURST, R. A., D. H. LANDERS, J. M. EILERS, D. F. BRAKKE, W. S. OVERTON, E. P. MEIER, AND R. E. CROWE. Characteristics of lakes in the Eastern United States. Volume 1. Population descriptions and physico-chemical relationships. U.S.E.P.A. Rep. 600/4-86/007a.
- MACKIE, G. L. 1989. Tolerances of five benthic invertebrates to hydrogen ions and metals (Cd, Pb, Al). *Arch. Environ. Contam. Toxicol.* 18: 215–223.
- MALLEY, D. F., AND P. S. S. CHANG. 1986. Increase in the abundance of Cladocera at pH 5.1 in experimentally-acidified Lake 223, Experimental Lakes Area, Ontario. *Water Air Soil Pollut.* 30: 629–638.
- MCDONALD, D. G., J. P. READER, AND T. R. K. DALZIEL. 1989. The combined effects of pH and trace metals on fish ionoregulation, p. 221–242. *In* R. Morris, E. Taylor, D. Brown, and J. Brown [ed.] *Acid toxicity and aquatic animals*. Cambridge University Press, Cambridge, MA.
- NALEWAJKO, C., AND B. PAUL. 1985. Effects of manipulations of aluminum concentrations and pH on phosphate uptake and photosynthesis of planktonic communities in two Precambrian Shield lakes. *Can. J. Fish. Aquat. Sci.* 42: 1946–1953.
- NEARY, B. P., P. J. DILLON, J. R. MUNRO, AND B. J. CLARK. 1990. The acidification of Ontario lakes: an assessment of their sensitivity and current status with respect to biological damage. *Tech. Rep.*, Ontario Ministry of the Environment, Dorset, Ont.
- NISSEN, J. P., OSTDAHL, AND W. T. W. POTTS. 1984. Species replacements in acidified lakes: physiology, predation of competition? *Inst. Freshwater Res. Rep.* 61: 148–153.
- PAGENKOPF, G. K. 1983. Gill surface interaction model for trace-metal toxicity to fishes: role of complexation, pH and water hardness. *Environ. Sci. Technol.* 17: 342–347.
- PINEL-ALLOUL, B., G. METHOT, G. VERREAULT, AND Y. VIGNEAULT. 1990. Zooplankton species associations in Quebec lakes: variation with abiotic factors, including natural and anthropogenic acidification. *Can. J. Fish. Aquat. Sci.* 47: 110–121.
- POTTS, W. T. W., AND G. FRYER. 1979. The effects of pH and salt content on sodium balance in *Daphnia magna* and *Acantholeberis curvirostris* (Crustacea: Cladocera). *J. Comp. Physiol.* 129: 289–294.
- SAS INSTITUTE. 1985. *SAS user's guide: statistics, version 5*. SAS Institute, Cary, NC.
- SIEGFRIED, C. A., J. A. BLOOMFIELD, AND J. W. SUTHERLAND. 1987. Analysis of plankton community structure in Adirondack lakes in relation to acidification, p. 445–450. *In* R. Perry, R. M. Harrison, J. N. B. Bell, and J. N. Lester [ed.] *Acid rain: scientific and technical advances*. Seiper Ltd., London.
- SPRULES, W. G. 1975. Midsummer crustacean zooplankton communities in acid-stressed lakes. *J. Fish. Res. Board Can.* 32: 389–395.
- SUTHERLAND, J. W. [ED.] 1989. Field surveys of the biota and selected water chemistry parameters in 50 Adirondack Mountain lakes. Final Report, Adirondack Biota Project, New York State Department of Environmental Conservation, Albany, NY.
- TESSIER, A. J., AND R. J. HORWITZ. 1990. Influence of water chemistry on size structure of zooplankton assemblages. *Can. J. Fish. Aquat. Sci.* 47: 1937–1943.
- YAN, N. D., AND W. GEILING. 1985. Elevated planktonic rotifer biomass in acidified metal-contaminated lakes near Sudbury, Ontario. *Hydrobiologia* 120: 199–205.
- YAN, N. D., AND R. STRUS. 1980. Crustacean zooplankton communities of acidic, metal-contaminated lakes near Sudbury, Ontario. *Can. J. Fish. Aquat. Sci.* 37: 2282–2293.

Effect of Short-Duration Seawater Exposure on Plasma Ion Concentrations and Swimming Performance in Coho Salmon (*Oncorhynchus kisutch*) Parr

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The effect of seawater (sw) on plasma ion concentrations and critical swimming velocity (U_{crit}) was investigated in hatchery-reared coho salmon (*Oncorhynchus kisutch*) parr exposed to one of four treatments: 24 h of seawater exposure (SW1), 5–7 d of seawater (SW5), 24 h in seawater followed by 24 h in fresh water (SW-FW), and a freshwater control (FWC). Only the SW1 fish demonstrated a reduced U_{crit} and, at rest, elevated plasma $[Na^+]$, $[Cl^-]$, and $[SO_4^{2-}]$. With exercise, SW1 fish were characterized by an increase in plasma ion concentrations and a decrease in both hematocrit (Hct) and muscle moisture content. There is a strong relationship between plasma $[Na^+]$ at rest and U_{crit} , where an optimal swimming velocity is obtained in animals with resting levels of approximately $147 \text{ mEq} \cdot L^{-1}$. Traditionally, the 24-h seawater challenge is used to test the hypoosmoregulatory ability in smolting salmonids, however, our data suggest that it may also predict the aerobic swimming potential of salmonids following seawater transfer. We suggest that the reduction in Hct and increase in plasma $[Na^+]$ result in reduced oxygen delivery to the muscle and that decrease in muscle moisture content impairs the contractile process.

L'effet de l'eau de mer sur les concentrations plasmatiques d'ions et la vitesse nataoire critique (U_{crit}) a été étudié chez des tacons de saumon coho (*Oncorhynchus kisutch*) élevés en éclosérie et exposés à l'un des quatre traitements suivants : exposition de 24 h à l'eau de mer (SW1), exposition de 5 à 7 jours à l'eau de mer (SW5), 24 h dans l'eau de mer puis 24 h dans l'eau douce (SW-FW), et un traitement témoin dans l'eau douce (FWC). Chez les poissons SW1, on a observé une réduction de U_{crit} et, au repos, des concentrations plasmatiques élevées de Na^+ , de Cl^- et de SO_4^{2-} . Lorsqu'ils étaient en mouvement, les poissons SW1 étaient caractérisés par une augmentation des concentrations plasmatiques d'ions et une diminution de l'hématocrite (Hct) et de la teneur musculaire en eau. Il existe une forte relation entre la concentration plasmatique de Na^+ au repos et U_{crit} , où la vitesse nataoire optimale est atteinte chez les animaux ayant des niveaux au repos d'environ $147 \text{ mEq} \cdot L^{-1}$. Traditionnellement, l'épreuve de 24 h dans l'eau de mer est utilisée pour mettre à l'essai la faculté hypo-osmorégulatrice des salmonidés en cours de smoltification; toutefois, nos données semblent indiquer qu'elle peut également prédire le potentiel nataoire aérobie des salmonidés après transfert dans l'eau salée. Nous pensons que la réduction de l'hématocrite et l'augmentation de la concentration plasmatique de Na^+ se traduisent par une diminution de l'apport d'oxygène dans les muscles et que la diminution de la teneur en eau des muscles altère le processus contractile.

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Salmon smolts moving from fresh water into an estuary are prone to extremely high mortality rates (Parker 1962; Durkin 1982; Healy 1982). Although the factors responsible for these high death rates are unknown, mortality could result from a variety of factors such as osmoregulatory stress, low food availability (Clarke 1982), predation (Larsson 1985), and other forms of competition. Individual survivorship is dependent on the ability of the challenged smolt to outperform inter- and intraspecific opponents. Migration into an estuary requires a dramatic change in osmoregulatory strategy (Eddy 1982), the success of which is dependent on the physiological status of the smolt.

The 24-h seawater challenge has frequently been used as a means to objectively quantify the hypoosmoregulatory condition of smolting salmonids before they are transferred to seawater net pens or released from hatcheries (Clarke and Blackburn 1977; Wedemeyer et al. 1980; Clarke 1982; Hogstrand and Haux 1985; Blackburn and Clarke 1987). Smolting

salmonids that exhibit large increases in plasma $[Na^+]$ following 24 h in full-strength seawater generally experience high mortality or survival with stunted growth following transfer to seawater net pens (Clarke and Shelbourn 1982). Clarke and Blackburn (1977) demonstrated in the laboratory that the greatest growth rates in salmonids transferred to seawater were achieved by those performing well in the seawater challenge. These data indicate that in a controlled environment, this test can be a reliable indicator of survival and growth potential following seawater transfer in salmonids. Little is known, however, about the effects of elevated ion levels on physical activities such as swimming ability which is fundamental to survival.

Rapid seawater transfer results in a reduction in sustained swimming performance in coho (*Oncorhynchus kisutch*) (Flagg et al. 1983; Smith 1987; Glova and McInerney 1977) and chum salmon (*O. keta*) (Houston 1959). The basis for this impairment has been speculated to be an osmoregulatory disturbance;

however, the only evidence to substantiate this is the work of Houston (1959) who found that the physical impairment following rapid seawater transfer in chum salmon was correlated with total body chloride and water levels. If the reduction in swimming performance is induced by an ionic imbalance detectable by ion analysis of the plasma, information obtained from a 24-h seawater challenge test may be used to estimate the degree to which swimming performance will be impaired in salmonids following transfer to seawater. Thus, the 24-h seawater challenge traditionally used to determine the hypoosmoregulatory status of a smolt may also be used as an indication of the animal's physical status and, therefore, chances for survival upon entry into a seawater environment such as an estuary.

This experiment was designed to test the hypothesis that elevated plasma ion concentrations, in particular Na^+ , result in a reduced ability for aerobic swimming in coho salmon. The parr-smolt transition is characterized by massive physiological adjustments, predisposing salmonids for marine survival while living in a freshwater medium (McCormick and Saunders 1987; Hoar 1988). Correlated with these changes is a large, transient reduction in swimming stamina in animals still in fresh water (Smith 1987) which is exacerbated by the seawater transfer. To examine the direct effect of elevated plasma ion concentrations on swimming performance independent of the reduction in swimming performance associated with smolting, the experiment was performed using presmolt coho salmon rapidly transferred to seawater. The physiological changes associated with this anticipated reduction in swimming performance were also determined.

Materials and Methods

Fish Acquisition and Care

Hatchery-reared coho salmon parr were obtained and transported from Spius Creek Hatchery, B.C., to the University of British Columbia. They were fed daily and held in dechlorinated fresh water at 10°C for 3 mo prior to experimentation. Mean length and weight were 10.1 ± 0.1 cm and 8.9 ± 0.2 g, respectively, during the test which was conducted between November 1989 and January 1990. Based on the visible parr marks and the season of the study, the fish used in the experiments are referred to as parr.

Experimental Procedure

Groups of 15 fish were randomly subjected to one of four treatments at 10°C : 24 h in natural seawater (26.5 ± 0.7 ppt) (SW1), 5–7 d in natural seawater (SW5) following 24 h in 50% seawater, 24 h in natural seawater followed by 24 h in fresh water (SW-FW), and a fresh water control (FWC). In all treatments, fish were rapidly transferred by dip net to the respective static water baths kept at constant temperature by placement in cooling troughs. The treatments were replicated six times over the duration of this study. Care was taken to ensure that fish densities during the various exposure regimes did not exceed $3.0 \text{ g}\cdot\text{L}^{-1}$ as is recommended by Blackburn and Clarke (1987). In this study, all parr survived the transfer to seawater.

Following each exposure regime, 10 of the 15 fish were placed in a modified Brett-type respirometer (Gehrke et al. 1990) filled with seawater (SW1 and SW5) or fresh water (SW-FW and FWC) and forced to swim for 2 h at a velocity of 3 body lengths (BL) $\cdot\text{s}^{-1}$. Subsequently, the critical swimming velocity (U_{crit}) was determined for each fish by subjecting the group to hourly $1\text{-BL}\cdot\text{s}^{-1}$ increases in water velocity until

all animals had fatigued. Fish were removed as they fatigued and individual U_{crit} s were calculated by adding the velocity of the most recently completed increment and the product of the proportion of the fatigue increment completed and the increase in velocity of that increment (Brett 1964). The proportion of the fatigue increment refers to the length of time the fish swam at the final velocity divided by 1 h, and the increase in velocity of the fatigue increment was $1 \text{ BL}\cdot\text{s}^{-1}$. No correction for the solid blocking effect of the fish was included in this calculation, as the total cross-sectional area of the fish did not exceed 5% of that of the swim tube. In these experiments, fatigue was defined as that point at which a fish could no longer remove itself from the posterior screen despite repeated prodding.

Individual U_{crit} s were determined for fish that swam in a group. The validity of this method has been criticized (Lindsey 1978) because schooling behaviour may result in nonfatigued fish drifting to the rear of the swim tunnel with other fatigued fish resulting in premature termination of the test by the researcher. In this study the choice was made to exercise fish in groups of 10, as Bams (1964) found no effect of schooling on fatigue in sockeye salmon (*Oncorhynchus nerka*) fry exercised in groups of 10–12 animals or less. An opaque plastic cover was placed at the anterior portion of the swim tunnel, enticing the salmon to remain there until the velocity could no longer be maintained and fatigue ensued. This prevented interactions with the posterior screen prior to exhaustion and ensured that animals were truly fatigued when they were removed. In all tests, the coho parr fatigued independently of one another and in an approximately normal distribution, suggesting that schooling behaviour has little influence on fatigue in this protocol. In addition, there was no apparent change in U_{crit} over the duration of the experiment in any of the treatment replications.

Sampling Procedure

Measurements in resting fish (rest) were obtained by terminal anaesthetization with MS 222 ($200 \text{ mg}\cdot\text{L}^{-1}$) by injecting a highly concentrated solution of the anaesthetic into a tank containing five animals isolated from their respective group at least 12 h prior to the U_{crit} determination. Anaesthetization prior to sampling has been demonstrated to have no effect on plasma ion concentrations (Blackburn and Clarke 1987). Exercised fish were killed either by concussion immediately following fatigue (U_{crit}) or by terminal anaesthetization after a 2-h recovery period (recovery). The latter technique was used in resting and recovering fish to minimize struggling associated with sampling and thus changes in muscle lactate concentration that would occur without anaesthesia. Terminal anaesthesia was not deemed necessary in fatigued fish because at this time, they were completely exhausted and could easily be removed and killed without the problems associated with struggling. Sampling consisted of recording fork length and weight, severing the caudal peduncle, and collecting blood in $60\text{-}\mu\text{L}$ microhematocrit tubes. The tubes were centrifuged at 11 500 rpm for 5 min in a Damon IEC MB microhematocrit centrifuge and the hematocrit (Hct) was measured in quadruplicate. Fish carcasses were frozen in liquid nitrogen within 2 min of death and the plasma and fish carcasses were stored at -80°C for later analyses.

Analytical Techniques

Muscle lactate concentrations were determined from 0.5 g of dorsal epaxial muscle. The muscle was homogenized in 3 mL

of ice-cold 0.6 N perchloric acid (PCA) for 15 s using a Kinnematica GmbH polytron. The homogenate was then centrifuged in a Sorvall Instruments RC5C refrigerated centrifuge at 13 000 rpm for 10 min at 2°C. One millilitre of the supernatant was placed in a 1.5-mL polypropylene Eppendorf micro test tube and neutralized with 0.1 mL of 3 M KHCO_3 and 0.5 M triethanolamine. This mixture was vortexed and placed in an Eppendorf centrifuge 3200 for 5 min. The supernatant was analyzed for lactate using a Sigma lactate assay kit (826-UV) and a Shimadzu UV-160 visible recording spectrophotometer.

Plasma ion concentrations were determined on a Shimadzu ion chromatograph (model HIC-6A) (IC) except in two of the six treatment replications where plasma sodium concentrations were determined on a Perkin-Elmer model 2380 atomic absorption spectrophotometer (aa). The plasma in microhematocrit tubes was thawed and in the case of the aa analyses diluted to within the linear range of the machine's detection. Preparation of plasma for the IC analysis consisted of adding 20 μL of plasma to 20 μL of methanol, for the purpose of deproteinization, and 60- μL of distilled deionized water in a 0.5-mL centrifuge tube. The contents were vortexed and spun in an Eppendorf centrifuge 3200 for 5 min. To measure monovalent cation concentrations, a 20- μL aliquot of the supernatant was injected into the IC equipped with a Shodex Y521 cation column and a 5 mM nitric acid mobile phase. A separate injection was required for anionic analysis which warranted the installation of a Shim-pak IC-A1 anion column to the IC and the use of a 6 mM boric acid, 18 mM mannitol, and 7.5 mM Tris (tris(hydroxymethyl)aminomethane) mobile phase.

Muscle moisture was determined on 0.5–1.0 g of dorsal epaxial muscle by expressing weight loss following desiccation as a function of initial wet weight of the tissue. Muscle was dried at 75–80°C to constant weight, approximately 96 h.

Statistics

Statistical differences between the treatment groups and physiological states were determined by a split plot, randomized complete block design ANOVA, followed by a Tukey test, with a probability level of 0.05 chosen as the limit of statistical significance. Correlation analyses were computed by least squares regression and tested for significance. All data are presented as mean $\pm$ standard error.

Results

Aerobic swimming performance, as indicated by U_{crit} , was significantly reduced in the SW1 group, while all other treatments had no effect on swimming performance (Fig. 1). The reduction in U_{crit} seen in SW1 is not solely a result of the seawater exposure at the time of U_{crit} as no effect on U_{crit} was seen in the 5-d acclimated animals that also swam in seawater. In the SW1 group, extracellular $[\text{Na}^+]$, $[\text{Cl}^-]$, and $[\text{SO}_4^{2-}]$ were significantly elevated relative to control (FWC) values at all sampling times (rest, fatigue, and recovery). In the SW1 group, ion concentrations at fatigue and recovery were all significantly elevated relative to the SW1 resting levels, with the exception of $[\text{SO}_4^{2-}]$ at fatigue (Fig. 2). Thus, the greatest deviations in extracellular ion concentrations within and between treatments are seen in fish with the lowest U_{crit} (SW1). In the SW5 group, the extracellular ions do not differ significantly from the FWC values until fatigue and recovery.

There is a reduction in U_{crit} as the plasma $[\text{Na}^+]$ increases in resting animals. This relationship is best represented by a second-order regression ($r^2 = 0.68$) that indicates that an opti-

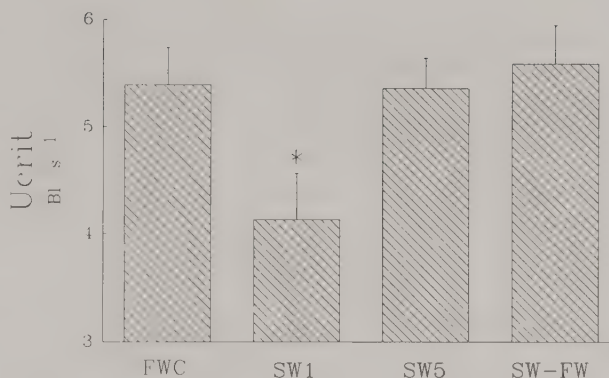


FIG. 1. Effect of salinity exposure regimes on critical swimming velocity (U_{crit}) of coho salmon parr. Treatments were as follows: FWC, freshwater control; SW1 and SW5, fish exposed to seawater for 1 and 5 d, respectively, prior to swimming in seawater; SW-FW, fish exposed to seawater for 1 d followed by 1 d in freshwater and U_{crit} determined in fresh water. The asterisk indicates a significant difference from the FWC group. In each treatment, six trials were performed, each consisting of 10 fish.

mal U_{crit} is attained in coho with a resting plasma $[\text{Na}^+]$ of 147.4 mEq·L⁻¹ (Fig. 3). There is a strong positive correlation ($r^2 = 0.89$) between the plasma $[\text{Na}^+]$ and $[\text{Cl}^-]$ measured in these animals at different activity levels following the various exposure regimes. For this reason, only the relationship between plasma $[\text{Na}^+]$ and U_{crit} is presented (Fig. 3) but a similar relationship also exists for $[\text{Cl}^-]$.

There are changes in both Hct and dorsal epaxial muscle moisture content, in addition to fluctuations in the concentrations of plasma ions following seawater exposure. At rest, there was no significant difference in Hct between treatments; however, at fatigue and following recovery, Hct in both groups that swam in seawater (SW1 and SW5) was significantly reduced relative to that measured at the same activity level in control (FWC) fish (Fig. 4). At recovery, Hct was significantly lower than the initial resting values in both seawater groups (SW1 and SW5).

The moisture content of the dorsal epaxial muscle was not significantly different between treatments in resting fish (Fig. 5). At fatigue, the only significant reduction in moisture content relative to that in control animals was in SW1 animals. Moisture content in the SW5 animals that swam in seawater does not differ significantly from control values at fatigue; however, during recovery, there is a significant reduction in content approaching that seen in SW1 animals. Thus, initially, SW5 animals are able to cope with the perturbation of elevated external salinity; however, this is not the case during recovery from exercise.

Lactate analysis of dorsal epaxial muscle was determined to quantify the relative contribution of anaerobiosis during the U_{crit} ; however, no significant differences between treatment conditions were detected (Fig. 5).

The SW-FW treatment was incorporated into the experimental design as an additional control for the FWC treatment. Due to the transfer into seawater, these animals experienced the same osmoregulatory perturbations and handling stresses as the SW1 animals and an additional transfer back into fresh water prior to swimming. In all parameters reported in this study, there were no significant differences between SW-FW and FWC treatments.

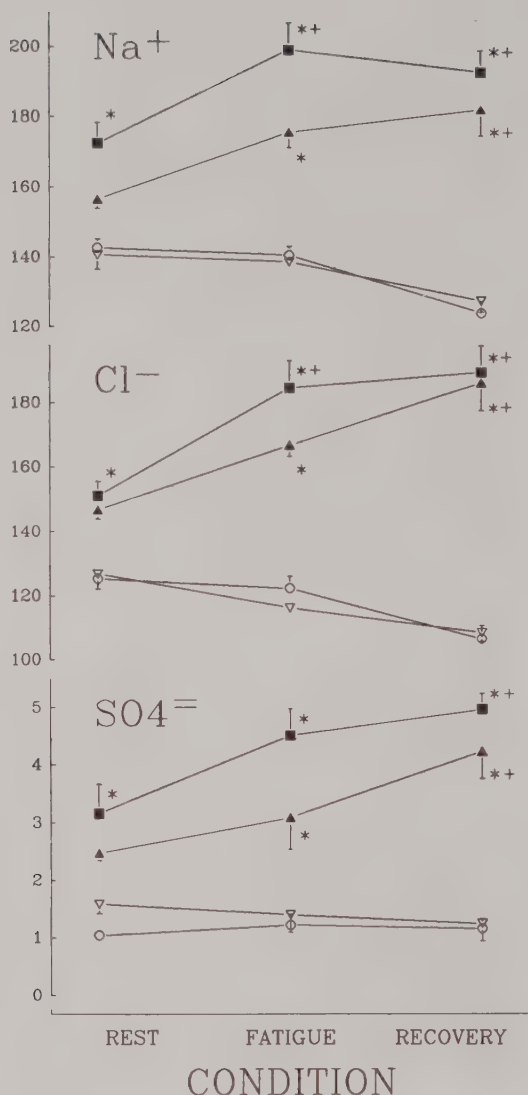


FIG. 2. Effect of treatment condition on plasma $[Na^+]$, $[Cl^-]$, and $[SO_4^{2-}]$ for four salinity exposure regimes: FWC ($\circ$), SW1 ($\blacksquare$), SW5 ($\blacktriangle$), and SW-FW (∇) (see Fig. 1 for further description). Rest, fatigue, and recovery refer to measurements taken before the fish began swimming, at U_{crit} , and 2 h following the U_{crit} determination, respectively. Asterisks indicate a significant difference relative to the same level of activity in FWC. Plus signs indicate a significant difference from the respective treatment resting value. In each treatment, six trials were performed, each consisting of five fish at each level of activity.

Discussion

The only condition to significantly reduce U_{crit} was exposure to seawater for 24 h (SW1) prior to exercise (Fig. 1). Transfer to seawater has been shown by others to impair swimming performance in coho smolts (Glova and McInerney 1977; Flagg et al. 1983; Smith 1987) and juvenile chum (Houston 1959). The SW1 group was characterized by significantly elevated plasma $[Na^+]$, $[Cl^-]$, and $[SO_4^{2-}]$ at all sample times relative to the FWC group (Fig. 2), indicating an ionic basis for the impairment in swimming performance.

The U_{crit} determination is predominantly an aerobic test. As a fish approaches fatigue, however, there is a sequential recruit-

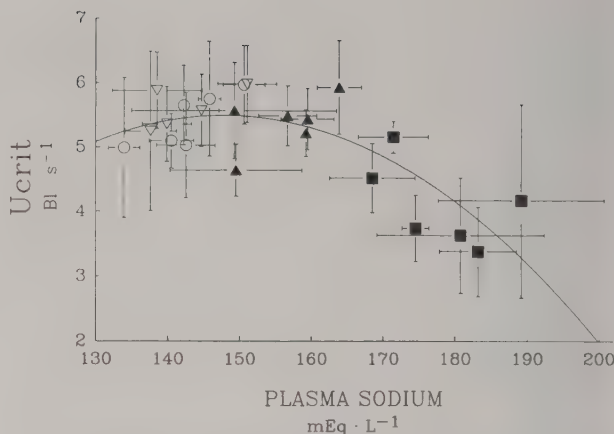


FIG. 3. Correlation between plasma $[Na^+]$ at rest and U_{crit} ($r^2 = 0.68$) (see Fig. 2 for symbol explanations).

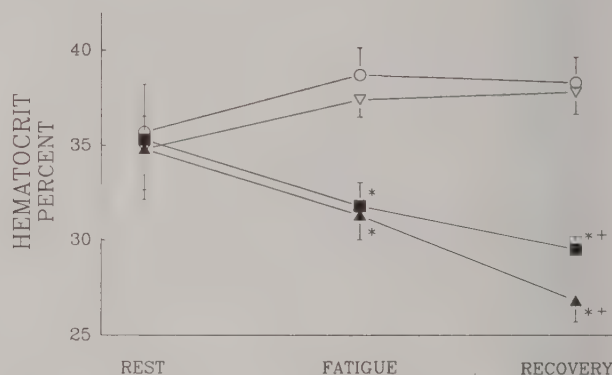


FIG. 4. Effect of treatment condition on Hct for four salinity exposure regimes (see Fig. 2 for other details).

ment of the anaerobic muscle fibres (Bone 1978; Bone et al. 1978) such that when the animal finally collapses, it is both aerobically and anaerobically exhausted. Anaerobic metabolism was unaffected by the various treatments as indicated by the similarity in muscle lactate concentrations following the various seawater exposure regimes (Fig. 5). Tang and Boutlier (1991) found rainbow trout (*Oncorhynchus mykiss*) acclimated to seawater to have higher muscle lactate at exhaustion and reduced lactate following recovery compared with fish exercised in fresh water, due to a greater clearance rate of lactate in the seawater animals. This was not found with seawater exposure in this study but these coho were only subjected to seawater for 5 d, while the rainbow trout used by Tang and Boutlier (1991) were acclimated for over 2 mo.

U_{crit} is strongly correlated with the plasma $[Na^+]$ of the resting animal (Fig. 3). The best-fit second-order regression suggests that an optimal swimming performance is achieved in animals with resting extracellular $[Na^+]$ levels of 147.4 mEq · L⁻¹. As $[Na^+]$ increases above this value, there is a reduction in U_{crit} . There is also a trend towards a reduction in U_{crit} as the $[Na^+]$ decreases below this value; however, ion levels were not depressed sufficiently in this study to show the effect unequivocally. The relationship between resting ion concentration and U_{crit} also exists for extracellular $[Cl^-]$, as there is a strong correlation between extracellular $[Cl^-]$ and $[Na^+]$.

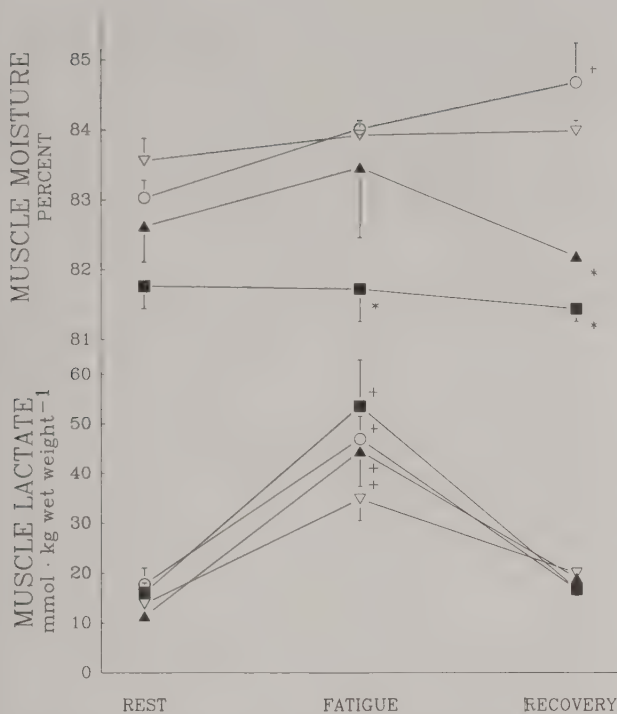


FIG. 5. Effect of treatment condition on percent muscle moisture and muscle lactate concentration for four treatment regimes (see Fig. 2 for other details).

This is in agreement with the work of Houston (1959) who found that sustained swimming performance was correlated with the total body $[Cl^-]$ at fatigue. These results imply that data acquired from a 24-h seawater challenge are of value in estimating a salmonid's ability to exercise, which has been used as an indicator of the overall condition of the animal.

Initially, 5 d appeared sufficient for the parr to compensate for the osmoregulatory stress associated with seawater transfer, as extracellular ion concentrations at rest in the SW5 group did not differ significantly from those in the FWC fish (Fig. 2) and U_{crit} was not affected by this treatment (Fig. 1). During recovery, however, the extracellular ion disturbance in the SW5 group was as severe as that observed in the SW1 group. This would indicate that in a second U_{crit} determination, the aerobic swimming ability may be equally impaired in both groups, if ion levels during recovery are indicative of exercise potential. Thus, SW5 animals were capable of initial compensation for the increased environmental salinity at rest and consequently were able to exploit their full aerobic capacity; however, during recovery the cost became apparent. Full adaptation to seawater in coho parr takes up to 30–35 d based on gill Na^+ , K^+ -ATPase activity (Zaugg and McLain 1970).

The reduced U_{crit} in group SW1 may be due to the poor hypoosmoregulatory ability; however, limitations to exercise may also exist at the level of the circulatory system or the muscle. In the circulatory system, the sites of impairment may be in the transfer of oxygen across the respiratory surface or in the transport and delivery of oxygen to the muscle by the blood. In the gills of rainbow trout immersed in 67% seawater in vitro, Bath and Eddy (1979a) demonstrated considerable dehydration of the secondary lamellae which could potentially explain the reduction in arterial PO_2 (Pa_{O_2}) seen in salmonids at rest during

seawater transfer. In rainbow trout resting in 67% seawater, this reduction in Pa_{O_2} lasts for over 10 d (Bath and Eddy 1979a), and in resting Atlantic salmon (*Salmo salar*) parr exposed to full-strength seawater, 48 h was not sufficient for recovery (Stagg et al. 1989). The degree to which Pa_{O_2} is reduced during exercise following seawater exposure, and the impact it will have on aerobic swimming performance, is questionable, as both groups SW1 and SW5 were exercised in seawater, while only the former experienced an impairment in locomotor activity; however, it may be that 5 d is sufficient for the coho parr to rehydrate the lamellae in seawater and restore blood oxygenation.

The transport and delivery of oxygen to the active tissues is also affected by seawater transfer. The Hct in the SW1 group decreased significantly with exercise relative to the freshwater groups, but the same trend was seen in the SW5 group (Fig. 4), demonstrating that the reduction in U_{crit} is not simply related to the reduction in Hct. A reduction in Hct in salmonids following seawater transfer has been attributed to dehydration of the erythrocytes (Bath and Eddy 1979a; Blackburn and Clarke 1987); however, it could also be due to an increased plasma volume, as rainbow trout have been demonstrated to greatly increase drinking rate (Eddy and Bath 1979) and reduce urination rate (Bath and Eddy 1979b) almost immediately following rapid transfer to 67% seawater. In smolting coho salmon, the reduction in Hct seen following seawater transfer is due to an increased plasma volume which results in a reduction in oxygen carrying capacity of the blood (C. J. Brauner, J. M. Shrimpton, and D. J. Randall, unpubl. data).

Although the decrease in Hct is not directly responsible for the reduction in U_{crit} , it may have exacerbated the effects of the increased plasma ion levels on the binding of oxygen to haemoglobin. Sauer and Harrington (1988) found that hyperchloremia in vitro reduced the haemoglobin's affinity for oxygen in sockeye salmon. The oxygen content of whole blood from white sucker (*Catostomus commersoni*) was significantly reduced in vitro following the addition of NaOH and HCl to approximate the strong ion difference seen in animals exposed to saline water (Walker et al. 1989); however, this reduction in oxygen content could have been induced partly by changes in pH. Thus, elevated plasma ions and a reduced Hct in concert may have limited oxygen delivery during exercise in the SW1 group.

An increased ionic composition of the blood may also influence oxygen delivery to the tissues through a reduction in cardiac output. Following NaCl injections in the toad *Bufo marinus* and the bullfrog *Rana catesbeiana*, Hillman (1984) observed that hypernatremia in excess of $170 \text{ mEq} \cdot L^{-1}$ reduced maximal cardiac output through a reduction in heart rate and stroke volume. In the toad, peak systolic pressure and cardiac contractility have also been shown to decrease following NaCl injections (Hillman and Withers 1988). The authors attributed the latter to a direct negative inotropic effect of hypernatremia and hyperchloremia on the cardiac muscle. If the salmonid cardiovascular system responds similarly to elevated extracellular ions, cardiac output and consequently the rate of oxygen delivery to the working muscles could be compromised in the SW1 group reducing U_{crit} .

The limitation to aerobic exercise in the SW1 group may also lie at the level of the muscle. Muscle moisture content of the SW1 group was significantly lower at fatigue and recovery than that of the groups exercised in fresh water (Fig. 5). This has been demonstrated in resting rainbow trout (Finstad et al. 1988) and chum fry exercising (Houston 1959) in seawater. Hyper-

osmotic infusions in dogs results in a reduction in muscle moisture content and an intracellular alkalosis (Makoff et al. 1970). Intracellular changes in pH will have an effect on muscular contraction through direct actions on enzyme activity, and a reduction in intracellular moisture content may affect ion and metabolite concentrations resulting in a deviation from optimal conditions for contractility.

Within each treatment, only the FWC group showed a significant change in muscle moisture from rest to recovery, increasing slightly in agreement with the observations of Wood and Randall (1973). U_{crit} was not affected in the SW5 group and muscle moisture did not differ significantly from the control group until recovery, further indicating that impairment in swimming performance would be seen in this group in a subsequent bout of aerobic exercise.

The elevated ionic composition of the plasma may have a direct effect on muscular contractility. Houston (1959) theorized that the reduced locomotory activity in chum salmon rapidly transferred to seawater was a direct result of altered electrolyte concentration on the neuromuscular apparatus. Homsher et al. (1974) observed a reduction in contractility following stimulation in frog sartorius muscle bathed in various hypertonic solutions. The authors attributed this to ionic disruption of contractile proteins. Thus, the direct action of elevated extracellular ions and the induced muscle water movements may also contribute to the locomotory impairment seen in the SW1 coho.

In conclusion, there is a reduction in swimming performance with an increased plasma $[Na^+]$ in response to a 24-h exposure to seawater (SW1) (Fig. 1). There is a strong correlation between the plasma $[Na^+]$ and $[Cl^-]$ measured at rest and the subsequent maximal aerobic swimming velocity. This implies that the seawater challenge test used as an indicator of seawater adaptability in smolting salmonids (Blackburn and Clarke 1987) may also be used as an indicator of the exercise potential of salmonids entering a seawater environment. The impairment in swimming performance seen in the SW1 group is related to the hypoosmoregulatory ability of the coho. The ionic composition of the plasma increases and there is a reduction in Hct and muscle moisture. The limitation to aerobic activity is postulated to exist at the level of the circulatory system, affecting oxygen delivery to the muscles, and at the cellular level of the muscle where a reduced moisture content leads to an impairment of contractility.

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References

- BAMS, R. A. 1964. Differences in performance of naturally and artificially propagated sockeye salmon migrant fry, as measured with swimming and predation tests. *J. Fish. Res. Board Can.* 24: 1117–1153.
- BATH, R. N., AND F. B. EDDY. 1979a. Ionic and respiratory regulation in rainbow trout during rapid transfer to seawater. *J. Comp. Physiol.* 134: 351–357.
- 1979b. Salt and water balance in rainbow trout (*Salmo gairdneri*) rapidly transferred from fresh water to sea water. *J. Exp. Biol.* 83: 193–202.
- BLACKBURN, J., AND W. C. CLARKE. 1987. Revised procedure for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. *Can. Tech. Rep. Fish. Aquat. Sci.* 1515: 35 p.
- BONE, Q. 1978. Locomotor muscle, p. 361–417. *In* W. S. Hoar and D. J. Randall [ed.] *Fish physiology*. Vol. 7. Academic Press, New York, NY.
- BONE, Q., J. KICENIUK, AND D. R. JONES. 1978. On the role of the different fibre types in fish myotomes at intermediate swimming speeds. *U.S. Fish Wildl. Serv. Fish. Bull.* 76: 691–699.
- BRETT, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Board Can.* 21: 1183–1226.
- CLARKE, W. C. 1982. Evaluation of the seawater challenge test as an index of marine survival. *Aquaculture* 28: 177–184.
- CLARKE, W. C., AND J. BLACKBURN. 1977. A seawater challenge test to measure smolting of juvenile salmon. *Can. Fish. Mar. Serv. Tech. Rep.* 705: 11 p.
- CLARKE, W. C., AND J. E. SHELBOURN. 1982. Growth and smolting of under-yearling coho salmon in relation to photoperiod and temperature. *Proc. North Pac. Aquacult. Symp.*, Aug. 1980, Anchorage, AK. Alaska Sea Grant Rep. 82-2: 200–216.
- DURKIN, J. T. 1982. Migration characteristics of coho salmon (*Oncorhynchus kisutch*) smolts in the Columbia River and its estuary, p. 365–376. *In* V. S. Kennedy [ed.] *Estuarine comparisons*. Academic Press, New York, NY.
- EDDY, F. B. 1982. Osmotic and ionic regulation in captive fish with particular reference to salmonids. *Comp. Biochem. Physiol.* 73B: 125–141.
- EDDY, F. B., AND R. N. BATH. 1979. Ionic regulation in rainbow trout (*Salmo gairdneri*) adapted to fresh water and dilute sea water. *J. Exp. Biol.* 83: 181–192.
- FINSTAD, B., M. STAURNES, AND O. B. REITE. 1988. Effect of low temperature on sea water tolerance in rainbow trout, *Salmo gairdneri*. *Aquaculture* 72: 319–328.
- FLAGG, T. A., E. F. PRENTICE, AND L. S. SMITH. 1983. Swimming stamina and survival following direct sea-water entry during parr-smolt transformation of coho salmon (*Oncorhynchus kisutch*). *Aquaculture* 32: 383–396.
- GEHRKE, P. C., L. E. FIDLER, D. C. MENSE, AND D. J. RANDALL. 1990. A respirometer with controlled water quality and computerized data acquisition for experiments with swimming fish. *Fish Physiol. Biochem.* 8: 61–67.
- GLOVA, G. J., AND J. E. MCINERNEY. 1977. Critical swimming speeds of coho salmon (*Oncorhynchus kisutch*) fry to smolt stages in relation to salinity and temperature. *J. Fish. Res. Board Can.* 34: 151–154.
- HEALY, M. C. 1982. Timing and relative intensity of size-selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. *Can. J. Fish. Aquat. Sci.* 39: 952–957.
- HILLMAN, S. S. 1984. Intropic influence of dehydration and hyperosmolar solutions on amphibian cardiac muscle. *J. Comp. Physiol. B* 154: 325–328.
- HILLMAN, S. S., AND P. C. WITHERS. 1988. The hemodynamic consequences of hemorrhage and hypernatremia in two amphibians. *J. Comp. Physiol. B* 157: 807–812.
- HOAR, W. S. 1988. The physiology of smolting salmonids, p. 275–343. *In* W. S. Hoar and D. J. Randall [ed.] *Fish physiology*. Vol. 11A. Academic Press, New York, NY.
- HOGSTRAND, C., AND C. HAUX. 1985. Evaluation of the sea-water challenge test on sea trout, *Salmo trutta*. *Comp. Biochem. Physiol.* 82A: 261–266.
- HOMSHER, E., F. N. BRIGGS, AND R. M. WISE. 1974. Effects of hypertonicity on resting and contracting frog skeletal muscles. *Am. J. Physiol.* 226: 855–863.
- HOUSTON, A. H. 1959. Locomotor performance of chum salmon fry (*Oncorhynchus keta*) during osmoregulatory adaptations to sea water. *Can. J. Zool.* 37: 591–605.
- LARSSON, P. O. 1985. Predation on migrating smolts as an important regulating factor for salmon, *Salmo salar*, populations. *J. Fish Biol.* 26: 391–397.
- LINDSEY, C. C. 1978. Form, function, and locomotory habits in fish, p. 1–100. *In* W. S. Hoar and D. J. Randall [ed.] *Fish physiology*. Vol. 7. Academic Press, New York, NY.
- MAKOFF, D. L., J. A. DA SILVA, B. J. ROSENBAUM, S. E. LEVY, AND M. H. MAXWELL. 1970. Hypertonic expansion: acid-base and electrolyte changes. *Am. J. Physiol.* 218: 1201–1207.
- MCCORMICK, S. D., AND R. L. SAUNDERS. 1987. Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth, and metabolism. *Am. Fish. Soc. Symp.* 1: 211–229.
- PARKER, R. R. 1962. Estimations of ocean mortality rates for Pacific salmon (*Oncorhynchus*). *J. Fish. Res. Board Can.* 19: 561–589.
- SAUER, J., AND J. P. HARRINGTON. 1988. Hemoglobins of the sockeye salmon, *Oncorhynchus nerka*. *Comp. Biochem. Physiol.* 91A: 109–114.
- SMITH, L. 1987. Improving hatchery effectiveness as related to smoltification. U.S. Dept. of Energy, Bonneville Power Administration, Division of Fish and Wildlife, May 1987. p. 97–108.

- STAGG, R. M., C. TALBOT, F. B. EDDY, AND M. WILLIAMS. 1989. Seasonal variations in osmoregulatory and respiratory responses to sea water exposure of juvenile atlantic salmon (*Salmo salar*) maintained in fresh water. *Aquaculture* 82: 219-228.
- TANG, Y., AND R. G. BOUTILIER. 1991. White muscle intracellular acid-base and lactate status following exhaustive exercise: a comparison between freshwater- and seawater-adapted rainbow trout. *J. Exp. Biol.* 156: 153-171.
- WALKER, R. L., P. R. H. WILKES, AND C. M. WOOD. 1989. The effects of hypersaline exposure on oxygen-affinity of the blood of the freshwater teleost *Catostomus commersoni*. *J. Exp. Biol.* 142: 125-142.
- WEDEMEYER, G. A., R. L. SAUNDERS, AND W. C. CLARKE. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. *U.S. Natl. Mar. Fish. Serv. Rev.* 42: 1-14.
- WOOD, C. M., AND D. J. RANDALL. 1973. The influence of swimming activity on water balance in the rainbow trout (*Salmo gairdneri*). *J. Comp. Physiol.* 82: 257-276.
- ZAUGG, W. S., AND L. R. McLAIN. 1970. Adenosinetriphosphatase activity in gills of salmonids: seasonal variations and salt water influence in coho salmon, *Oncorhynchus kisutch*. *Comp. Biochem. Physiol.* 35: 587-596.

Influence of Fat Content on Uptake and Depuration of the Off-flavor 2-Methylisoborneol by Channel Catfish (*Ictalurus punctatus*)

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Channel catfish (*Ictalurus punctatus*) of different tissue fat contents (0.5–11.0%) were held in water containing approximately 0.5 µg/L of the off-flavor compound 2-methylisoborneol (MIB) for various times. A new analytical method was developed to quantify tissue burdens of MIB. Fish showed significant bioconcentration of MIB after 2 h and equilibrium by 24 h. The fatter fish (>2.5% muscle fat) accumulated nearly three times more MIB than lean fish (<2%). Purging fish in MIB-free water indicated that leaner fish depurate faster (8 h) than fatter fish (48 h). Fat content had more influence in determining the time for return to acceptable flavor than initial MIB concentration. Managing catfish production to harvest leaner fish could minimize the impact of off-flavor on fish producers.

Des barbus de rivière (*Ictalurus punctatus*) présentant différentes teneurs tissulaires en graisses (de 0,5 à 11,0%) ont été détenues dans de l'eau contenant environ 0,5 µg/L du composé 2-méthylisobornéol (MIB) générant une saveur désagréable pendant différentes périodes. Une nouvelle méthode analytique a été mise au point pour quantifier la charge tissulaire en MIB. On a noté chez les poissons une bioconcentration significative de MIB après 2 h et un équilibre à 24 h. Les poissons plus gras (graisse musculaire > 2,5%) ont accumulé près de trois fois plus de MIB que les poissons maigres (< 2%). La purge des poissons dans de l'eau ne contenant pas de MIB a indiqué que les poissons plus maigres détoxifient plus rapidement (8 h) que les poissons plus gras (48 h). La teneur en graisses a eu plus d'influence pour déterminer le temps requis jusqu'au retour à une saveur acceptable que la concentration de MIB initiale. La gestion de la production de la barbe de rivière avec capture des poissons plus maigres seulement pourrait minimiser l'impact de la saveur désagréable sur les producteurs de poisson.

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Accumulation of environmentally derived off-flavors by farm-raised fishes is a major economic problem confronting the aquaculture industry (Tucker and Martin 1991). Fish with off-flavors are unmarketable and must be held and fed until the objectional flavors disappear. This is costly to the farmer in terms of interrupted cash flow, increased exposure to disease and loss by occasional oxygen deprivation, and lower prices paid for fish exceeding the optimum size. In addition, holding off-flavor fish prohibits restocking for future crops.

Geosmin (*trans*-1,10-dimethyl-*trans*-decalol; GSM) and 2-methylisoborneol (MIB) are produced by blue-green algae and actinomycetes which thrive in the nutrient-enriched ponds (Johnsen and Kuan 1987). Channel catfish (*Ictalurus punctatus*) (Lovell et al. 1986; Martin et al. 1987), rangia clams (Hsieh et al. 1988), and penaeid shrimp (Lovell and Broce 1985) containing these compounds were unacceptable to consumers because of earthy/musty off-flavors.

To date, efforts to control off-flavors using biocontrol agents and selective herbicides have been relatively unsuccessful (Tucker and Lloyd 1985, 1987). The most effective management strategy is to hold fish in production ponds until they regain acceptable flavour quality or, in some extreme cases,

transfer fish to special purging ponds for elimination of off-flavors.

However, rates of off-flavor uptake and depuration and the factors which effect them are not well understood. Lovell and Sackey (1973) demonstrated that fish exposed to algae producing an off-flavor became tainted within 1 d and reached maximum intensity in 10 d. In the laboratory, catfish fingerlings bioconcentrate 10-fold greater than found in the water, with maximal uptake occurring in less than 2 h (Martin et al. 1988). Preliminary work by Johnsen (1989) also indicated that both GSM and MIB are rapidly concentrated from the water. Fish became severely off-flavor in as little as 2 h but continued to accumulate off-flavor compounds through a 24-h exposure period. Martin et al. (1990) reported that MIB injected directly into the circulatory system was rapidly transferred to various fatty tissues but also rapidly disappeared from the fish. However, specific rates of uptake and depuration by food-size fish of approximately 0.5 kg have not been investigated.

Knowledge of the kinetics of off-flavor uptake and depuration would be useful in evaluating the efficacy of off-flavor abatement treatments and management strategies.

To determine the role of fat content on the kinetics of MIB flux through food-size channel catfish, fish of various tissue fat contents were exposed to solutions of MIB for different times.

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Additionally, exposed fish were held in clean (MIB-free) water for purging. Body burdens of MIB were measured using a newly developed instrumental technique.

Materials and Methods

Preparation of MIB

MIB was synthesized from *d*-camphor by the method of Wood and Snoeyink (1977). Briefly, *d*-camphor was reacted with methylmagnesium iodide by a Grignard reaction to produce MIB. The unreacted camphor was derivatized with alkaline hydroxylamine to produce a camphor oxime. This impurity was then extracted with 15 washes of sodium hydroxide. Purity of the remaining material was determined by combined gas chromatography and mass spectrometry. The mass spectrum matched the National Bureau of Standards reference for MIB (CAS No. 2371-42-8) with its characteristic molecular ion at *m/e* 168 and a very strong base peak at *m/e* 95. We estimate that the crystals are 94.9% pure and contaminated with *d*-camphor oxime (2.8%) and isoborneol (2.3%). Stock solutions of MIB were prepared by dissolving crystals in ethanol (499 g/L) and diluting with water to 1.25 µg/L.

Uptake and Depuration Trials

Fish with varying tissue fat contents (estimated by size and condition factor) were held for 14 d in flowing charcoal-filtered city water to purge any off-flavors. During this acclimation period, fish were not fed and were, therefore, in a fasting state.

For each trial, 12 fish (500 ± 100 g) were transferred to each of five 70-L test tanks. Water flow rate into each tank was 0.5 L/min and temperatures were regulated ($25.5 \pm 1^\circ\text{C}$) by opposing heating and cooling. A constant-delivery exposure system was chosen because preliminary trials indicated that volatile loss of MIB from water was approximately 50% in 24 h. The stock solution of MIB was metered into the head tank with a peristaltic pump for a desired concentration of 1.0 µg/L in the exposure tanks. However, determinations of MIB concentrations made throughout the trial using the method of Johnsen and Kuan (1987) indicated a lower than desired concentration of 0.44 ± 0.1 µg/L during exposures. At the transition between exposure and depuration, both exposure and head tanks were drained 90% and refilled to effect a rapid reduction in MIB concentration. After 1 h of depuration, MIB concentrations in water samples were below instrumental detection (0.1 µg/L).

In the uptake and depuration trials, fish were sampled at 0, 2, 4, 8, and 24 h of exposure and 8, 24, 48, and 72 h of depuration. Three fish were collected at each sampling time, headed, gutted, and frozen.

Sample Preparation and Fat Analysis

To prepare fish samples for determination of MIB burdens, fish were thawed at 4°C and skinned using a Jacard A35-P membrane skinner (Buffalo, NY). This commercial skinning technique removes the skin but leaves subcutaneous fat. The fish were hand-filletted and belly flaps were removed to produce a commercial shank fillet. Fat content of the fillets was determined by the perchloric-acetic acids method of Konecko (1985).

Chemical Analysis

Previous methods for the quantification of MIB concentrations (Martin et al. 1987; Johnsen et al. 1992) were not suffi-

ciently reliable for these experiments. Therefore, an improved method was developed.

To extract MIB from the tissue, 50 g of the fillet sample was added to 750 mL of distilled and deionized water. The mixture was homogenized in a blender and then added to a 1-L boiling flask. An internal standard (IS) of 100 µL of 25 µg undecanone/L in hexane was added to the sample. The sample was then sparged with nitrogen and trapped under house vacuum (74.5 kPa) for 3 h.

The traps were constructed from 83×7 mm (inside diameter) glass tubes containing Carbopack B (300 mg) and Carbo-sieve III (100 mg) (Sulpeco Inc. Bellefonte, PA) separated by glass wool plugs (Fig. 1). Traps were washed with 10 mL of hexane before each experiment. The sample flasks were immersed in a 80°C water bath; the sample was sparged with nitrogen (500 mL/min) during the trapping period. To elute the sample, 10 mL of hexane was forced through the trap. This was concentrated under nitrogen to 50 µL and sealed in an autosampler vial.

Concentrations of MIB were determined using gas chromatography and mass spectrometry. A Hewlett Packard (Palo Alto, CA) 7673A autosampler injected 1 µL of sample into a 5890 gas chromatograph equipped with a $50 \text{ m} \times 0.2 \text{ mm}$ (5% phenylmethyl silicone) column. The injector temperature was 200°C , while the oven temperature was held at 35°C for 5 min. The temperature was ramped at $2.5^\circ/\text{min}$ to 200°C followed by a $10^\circ/\text{min}$ ramp to 250°C . The temperature was held at 250°C for 11 min for column cleanup. Temperature of the transfer line between the gas chromatograph and mass spectrometer was 280°C .

A Hewlett Packard 5970 mass spectrometer using Selective Ion Monitoring (SIM) of masses 95.1 and 168 for compound identity confirmation was used to establish area counts. Concentration curves and single point calibrations made during each trapping experiment allowed concentrations to be calculated in micrograms per kilogram.

Five experimental fish samples spiked with the undecanone IS and three recovery standard samples were trapped in each session. These three concentration standards of 10 and 20 µg MIB/kg in water and a water blank spiked with the IS were analyzed.

Reproducibility

The suitability of the method to quantify both GSM and MIB was determined. Because GSM has a similar flavor to MIB, it was also assayed during the preexposure purging periods. Solutions of water or vegetable oil with known amounts of MIB, GSM, and the IS were used to measure the variations due to traps and elution efficiency. Analyses indicated that these factors introduced noise rather than bias and thus had a negligible effect on the results ($<1\%$ coefficient of variation). Experiments with 370 g of spiked fish tissue established the reproducibility of the technique. This sample was subdivided for five repeated measures and indicates that the coefficient of variation is less than 7%.

Efficiency, Sensitivity, and Calibration

The efficiency of the technique was determined by analyses of spiked fish samples. MIB (80 µL of 25 µg/kg), GSM (80 µL of 25 µg/kg), and IS (100 µL of 25 µg/kg) were added to fish homogenates. Calculations of recovery efficiency were based on quantification of direct-injected authentic compounds. Efficiency for recovery was 38.9% for MIB, 59.0% for GSM, and 83.1% for IS.

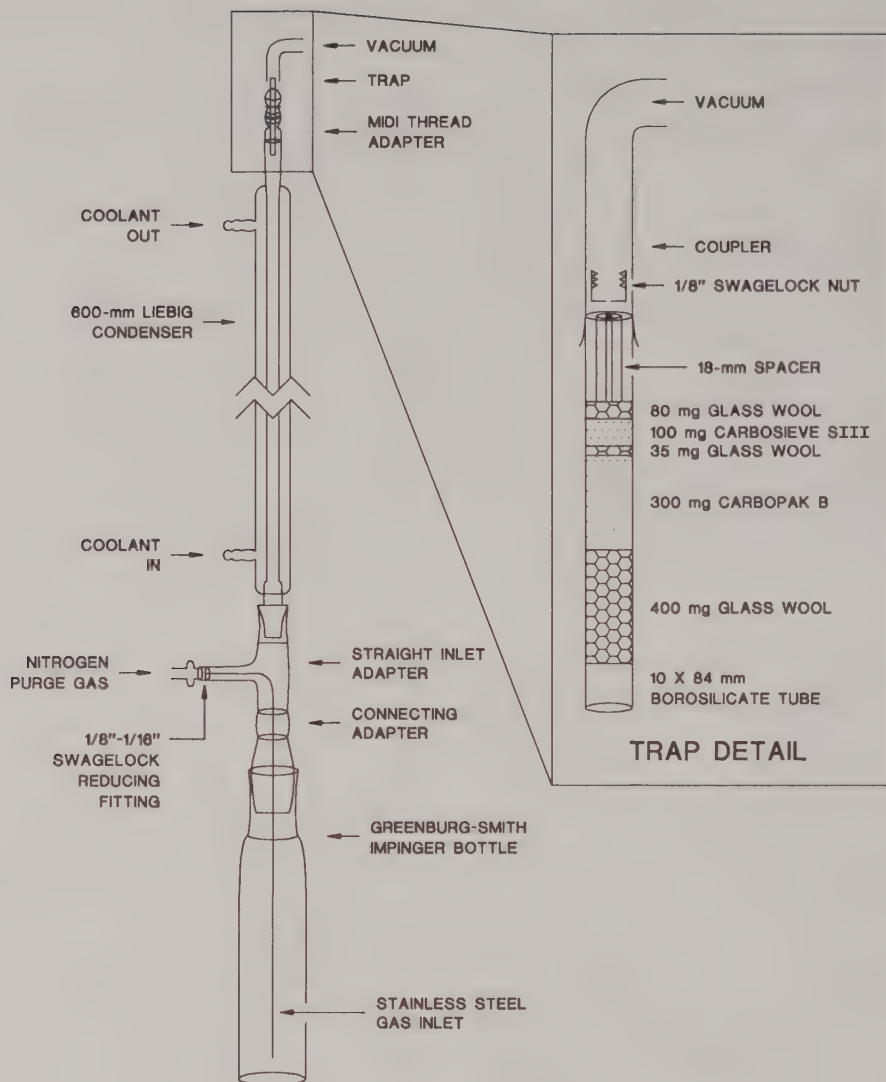


Fig. 1. Purge and trap distillation apparatus with detail of trap construction.

Standard curves of direct injections of GSM and MIB were used to establish linearity of the detectors. A concentration series of spiked fish was trapped and analyzed to determine linearity (slope = 1.03; $r = 0.89$) and sensitivity of the technique. Using SIM, the detection limit for both MIB and GSM was established at 0.1 $\mu\text{g/kg}$ in a 50-g sample with valid quantification above 0.5 $\mu\text{g/kg}$.

Results and Discussion

Uptake of MIB

The fish bioconcentrated MIB from water and became significantly ($p < 0.01$) off-flavor within the first 2 h and continued to accumulate MIB throughout the 24-h exposure period (Fig. 2). This was confirmed by taste tests. Fish exposed to MIB for up to 96 h showed no further accumulation ($p < 0.05$) beyond the first 24-h period (Fig. 3). These observations differ from those of Martin et al. (1988) who

reported maximal uptake in less than 2 h for 5 and 50 $\mu\text{g/L}$ concentrations of MIB in the water and much smaller fish. We used lower exposure concentrations and larger fish than Martin et al. (1988) which may account for the contradictory findings.

Fat content in the experimental fish varied from 0.5 to 11.0% and had a significant effect on the uptake of MIB. Lean fish bioconcentrated less MIB during a 24-h exposure than the fatter fish (Fig. 2). After 24 h of exposure, MIB concentrations were 7.4, 8.0, and 8.3 $\mu\text{g/kg}$ for fish with fat contents of 0.5, 1.0, and 1.5% respectively. Fish with fat contents of 2.5, 4.0, and 6.0% exhibited MIB concentrations of 20.1, 24.3, and 20.8 $\mu\text{g/kg}$, respectively. Assignment of fish to lean and fat fish categories was based on evaluation of the data following the experiment.

Regression analyses (SAS Inc., Cary, NC) showed that a strong positive correlation existed between MIB concentrations and fat content (Fig. 4) after only 2 h of exposure and increased through 24 h. However, lean fish had a higher mean concentration of MIB per gram of fat than the fatter fish (e.g.

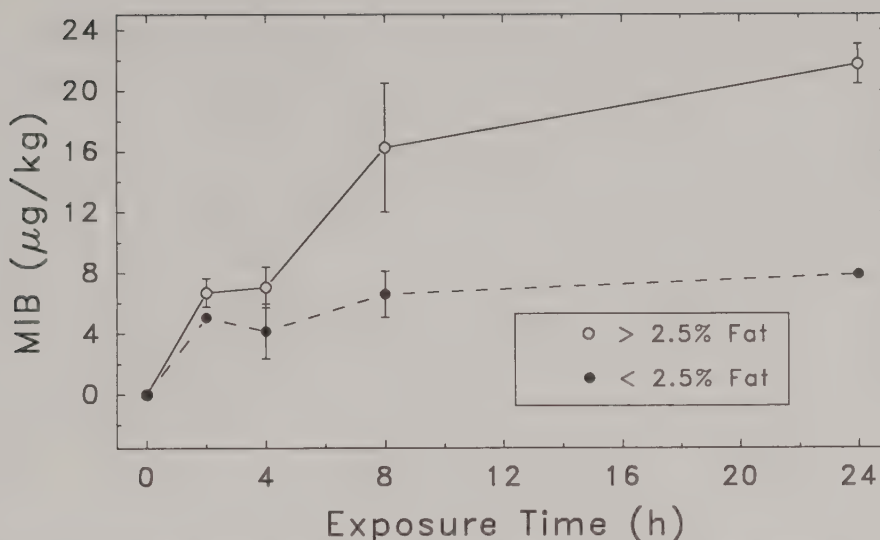


FIG. 2. The 24-h uptake of MIB by channel catfish (means $\pm$ SE, $n = 3$ for each measure).

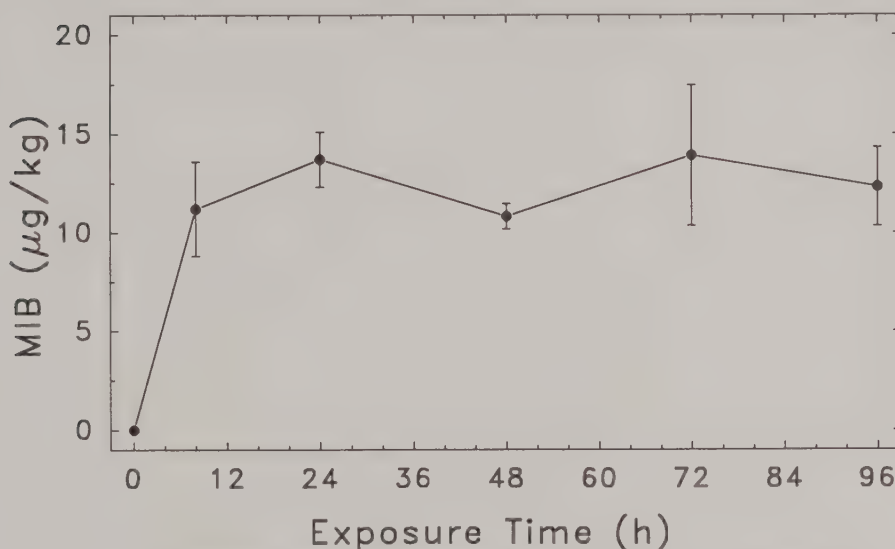


FIG. 3. The 96-h uptake of MIB by channel catfish (mean $\pm$ SE, $n = 3$ for each measure).

0.89 versus 0.57 $\mu\text{g/g}$ fat) at 24 h, indicating that the leaner fish did not reach a MIB equilibrium or saturation level at these concentrations.

Models of organic chemical accumulation in fishes indicate that bioconcentration directly from the water is, by far, more important to bioaccumulation than biomagnification through the dietary route (Schuurmann and Klein 1988). Lovell and Sackey (1973) demonstrated that, while catfish exposed to algae producing off-flavor do ingest some of the algae, they become off-flavor just as rapidly in filtered water where there is no physical contact and ingestion of the algae. Experimental exposures of rainbow trout (*Oncorhynchus mykiss*) to water containing GSM-producing algae demonstrated that off-flavor was acquired most rapidly through the gills followed by slower rates through the skin, small intestine, and stomach (From and Horlyck 1984).

A compound's hydrophobicity, which is directly related to the molecule's structure, is generally accepted to be the driving

force for bioaccumulation (Schuurmann and Klein 1988). The extreme lipophilic nature of MIB suggests that its uptake by channel catfish is most probably across gill membranes during respiration. This brings off-flavor metabolites into contact with lipid-containing blood which then distributes the compounds to other tissues.

Depuration of MIB

Purging of MIB was monitored in catfish placed into clean water following 24-h exposures to MIB. Concentrations were much reduced after just 8 h of purging and continued to decline through 24 h (Fig. 5). In the lean fish (fat content $< 2\%$), MIB concentrations dropped from a mean of 7.9 $\mu\text{g/kg}$ to below 1.0 $\mu\text{g/kg}$ after 8 h of purging and to zero detectable levels by 48 h. In fish with fat contents $> 2\%$, MIB remained detectable (0.5–0.7 $\mu\text{g/kg}$) after 48 h of purging. Persson (1980) reported that the sensory detection threshold for MIB in fish tissue ranged from 0.07 to 0.55 $\mu\text{g/kg}$. Similarly, our own sensory panels

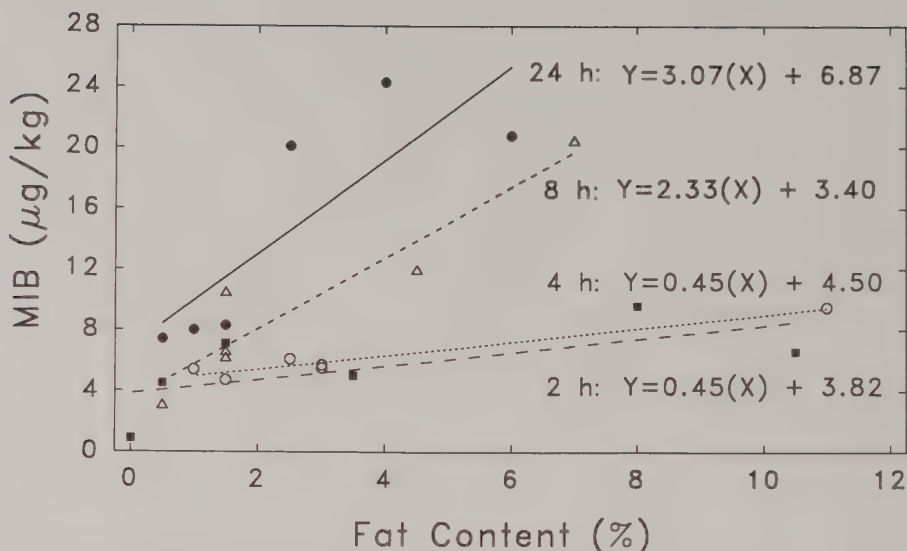


FIG. 4. Linear regressions of fat content versus MIB concentration (2 h: $r^2 = 0.94$; 4 h: $r^2 = 0.44$; 8 h: $r^2 = 0.89$; 24 h: $r^2 = 0.69$).

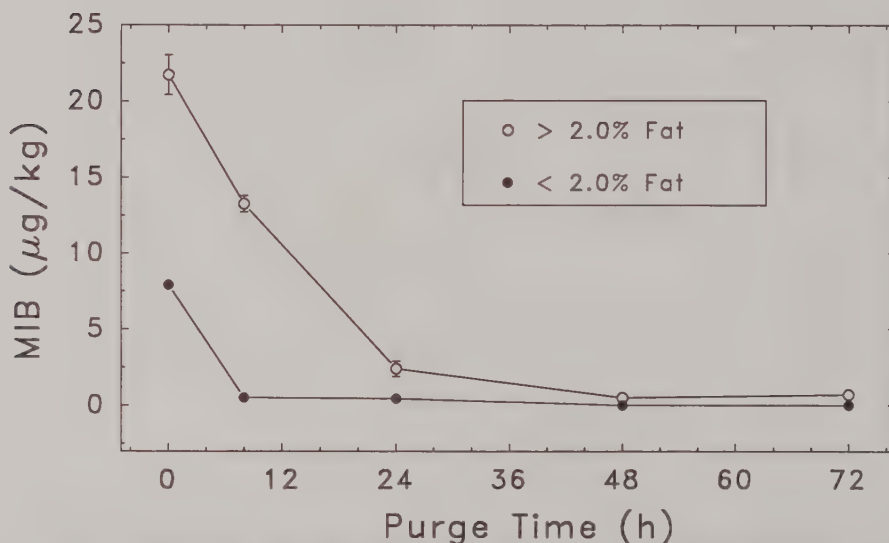


FIG. 5. Depuration of MIB from channel catfish (mean $\pm$ SE, $n = 3$ for each measure).

(Johnsen and Kelly 1990) reported detectable MIB flavors at $0.7 \mu\text{g/kg}$ but no off-flavors in samples with zero detectable MIB levels.

The residual level of MIB following purging depended more on fat content than starting MIB concentrations. The initial rate of depuration was similar for both fat and lean fish. However, lean fish reached a mean of $0.45 \mu\text{g/kg}$ after 24 h of purging and nondetectable levels after 48 h, while fatter fish retained a mean concentration of $0.5 \mu\text{g/kg}$ at 48 and 72 h. The much smaller and thus presumably leaner fingerlings of Martin et al. (1988) did purge to zero from much higher initial tissue concentrations of 32 and $320 \mu\text{g/kg}$, supporting this hypothesis.

The mechanisms by which MIB is lost from the tissues of catfish is not known. Martin et al. (1990) suggested that elimination via the gills or rapid biotransformation may have

accounted for their observations of rapid losses of MIB in all tissue compartments examined. Transformation of MIB into the dehydration products 2-methylenebornane and 2-methyl-2-bornene, reported as off-flavor compounds found in catfish (Martin et al. 1988), was ruled out because these compounds were never observed in our experiments. However, removal of MIB from lipid storage reserves and repartitioning into the water across the gill surface seems unlikely. Therefore, the actual mechanism remains to be determined.

The relationship of MIB uptake and depuration with fat content would suggest that attempts to produce leaner fish would be rewarded by lower levels of off-flavor compounds and more rapid purging times. Processed fish average 7% fat (Nettleton et al. 1990), which is in the range of our fat fish. Therefore, fish breeding, nutrition, and management programs to develop

leaner fish, within practical limits, could lessen the impact of the off-flavor problem.

References

- FROM, J., AND V. HORLYCK. 1984. Sites of uptake of geosmin, a cause of earthy-flavor, in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 41: 1224–1226.
- HSIEH, T. C.-Y., U. TANCHOTIKUL, AND J. E. MATIELLA. 1988. Identification of geosmin as the major muddy off-flavor of Louisiana brackish water clam (*Rangia cuneata*). J. Food. Sci. 53: 1228–1229.
- JOHNSEN, P. B. 1989. Factors influencing the flavor quality of farm-raised catfish. Food Technol. 43: 94–97.
- JOHNSEN, P. B., H. P. DUPUY, M. G. LEGENDRE, AND G. J. FLICK. 1992. Instrumental analysis of farm-raised catfish flavor quality, p. 361–376. In G. J. Flick and R. E. Martin [ed.] Advances in seafood biochemistry: composition and quality. Technomic Publishing Co. Inc., Lancaster, PA.
- JOHNSEN, P. B., AND C. A. KELLY. 1990. A technique for the quantitative sensory evaluation of farm-raised catfish. J. Sens. Stud. 4: 189–199.
- JOHNSEN, P. B., AND J. W. KUAN. 1987. Simplified method to quantify geosmin and 2-methylisoborneol concentrations in water and microbiological cultures. J. Chromatogr. 409: 337–342.
- KONIECKO, E. S. 1985. Handbook of meat analysis. Avery Publishing Group, Inc., Wayne, NJ. 289 p.
- LOVELL, R. T., AND D. BROCE. 1985. Cause of musty flavor in pond-cultured penaeid shrimp. Aquaculture 50: 169–174.
- LOVELL, R. T., I. Y. LELANA, C. E. BOYD, AND M. S. ARMSTRONG. 1986. Geosmin and musty-muddy flavors in pond-raised channel catfish. Trans. Am. Fish. Soc. 115: 485–489.
- LOVELL, R. T., AND L. A. SACKKEY. 1973. Absorption by channel catfish of earthy-musty flavor compounds associated with blue-green algae. Trans. Am. Fish. Soc. 102: 774–777.
- MARTIN, J. F., L. W. BENNETT, AND W. H. GRAHAM. 1988. Off-flavor in the channel catfish (*Ictalurus punctatus*) due to 2-methylisoborneol and its dehydration products. Water Sci. Technol. 20: 99–105.
- MARTIN, J. F., C. P. MCCOY, W. GREENLEAF, AND L. BENNETT. 1987. Analysis of 2-methylisoborneol in water, mud, and channel catfish (*Ictalurus punctatus*) from commercial culture ponds in Mississippi. Can. J. Fish. Aquat. Sci. 44: 909–912.
- MARTIN, J. F., S. M. PLAKAS, J. H. HOLLEY, AND J. V. KITZMAN. 1990. Pharmacokinetics and tissue disposition of the off-flavor compound 2-methylisoborneol in the channel catfish (*Ictalurus punctatus*). Can. J. Fish. Aquat. Sci. 47: 544–547.
- NETTLETON, J. A., W. H. ALLEN, JR., L. V. KLATT, W. M. N. RATNAYAKE, AND R. G. ACKMAN. 1990. Nutrients and chemical residues in one-two pound Mississippi farm-raised channel catfish (*Ictalurus punctatus*). J. Food Sci. 55: 954–958.
- PERSSON, P.-E. 1980. Sensory properties and analysis of two muddy odour compounds, geosmin and 2-methylisoborneol, in water and fish. Water Res. 14: 1113–1118.
- SCHUURMANN, G., AND W. KLEIN. 1988. Advances in bioconcentration prediction. Chemosphere 17: 1551–1574.
- TUCKER, C. S., AND S. W. LLOYD. 1985. Evaluation of a commercial bacterial amendment for improving water quality in channel catfish ponds. Miss. Agric. For. Exp. Stn. Res. Rep. Vol. 10, No. 9: 3 p.
1987. Evaluation of potassium ricinoleate as a selective blue-green algicide in channel catfish ponds. Aquaculture 65: 141–148.
- TUCKER, C. S., AND J. F. MARTIN. 1991. Environmental related off-flavors in fish, p. 133–179. In J. R. Tomaso and D. Brune [ed.] Water quality in aquaculture. World Aquaculture Books, Baton Rouge, LA.
- WOOD, N. F., AND V. L. SNOEYINK. 1977. 2-methylisoborneol, improved synthesis and quantitative gas chromatography method for trace concentrations producing odor in water. J. Chromatogr. 132: 405–420.

Bacterial Productivity in Forested and Open Streams in Southern Ontario

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Hudson, J. J., J. C. Roff, and B. K. Burnison. 1992. Bacterial productivity in forested and open streams in southern Ontario. *Can. J. Fish. Aquat. Sci.* 49: 2412–2422.

Bacterial abundance, biomass, and heterotrophic production were measured in the water, sediment, and epilithon of forested and open streams in southern Ontario in summer 1988. Relationships of environmental variables to production were examined. The time course of nucleoside incorporation, recovery efficiency of bacterial DNA, isotope dilution, and disturbance artifacts were examined to compare bacterial production rates and to determine the appropriateness of the rate of [³H]thymidine incorporation into bacterial DNA as an estimate of bacterial production in these habitats. Water column bacterial biomass (12–97 $\mu\text{g C}\cdot\text{L}^{-1}$) and heterotrophic production (0.21–67 $\mu\text{g C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$) were greater in open streams than in forested streams. Differences between open and forested stream sediment bacterial biomass (0.30–1.1 $\text{g C}\cdot\text{m}^{-2}$) and heterotrophic production (18–140 $\text{mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) were not as pronounced as they were in the water column. A methodological disturbance artifact may have introduced a minor bias in sediment production measurements. Epilithic bacterial biomass was 35–150 $\text{mg C}\cdot\text{m}^{-2}$, and heterotroph production was 1.3–51 $\text{mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, significantly greater ($P < 0.05$) in open streams than in forested streams. Epilithic production and stream water temperature were positively correlated ($P < 0.05$). Heterotrophic bacterial production exceeded net primary production in forested streams, but not in open streams.

L'abondance bactérienne, la biomasse et la production hétérotrophe ont été mesurées dans l'eau, les sédiments et l'épilithon de cours d'eau à couvert forestier et de cours d'eau à découvert dans le sud de l'Ontario au cours de l'été 1988. On a examiné les relations entre les variables du milieu et la production. On a étudié le temps d'incorporation des nucléosides, l'efficacité de récupération de l'ADN bactérien, la dilution des isotopes et les artefacts de perturbation afin de comparer les taux de production bactérienne et de déterminer s'il est approprié d'utiliser le taux d'incorporation de [³H]thymidine à l'ADN bactérien à titre d'estimation de la production bactérienne dans ces habitats. La biomasse bactérienne de la tranche d'eau (12 à 97 $\mu\text{g C}\cdot\text{L}^{-1}$) et la production hétérotrophe (0,21 à 67 $\mu\text{g C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$) étaient plus élevées dans les cours d'eau à découvert que dans les cours d'eau à couvert forestier. Dans le cas des sédiments des cours d'eau à découvert et à couvert forestier, les différences entre la biomasse bactérienne (0,30 à 1,1 $\text{g C}\cdot\text{m}^{-2}$) et la production hétérotrophe (18 à 140 $\text{mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) n'étaient pas aussi prononcées qu'elles ne l'étaient dans la tranche d'eau. Un artefact de perturbation méthodologique peut avoir introduit une erreur systématique mineure dans les mesures de la production des sédiments. Dans l'épilithon, la biomasse bactérienne était de 35 à 150 $\text{mg C}\cdot\text{m}^{-2}$ et la production hétérotrophe était comprise entre 1,3 et 51 $\text{mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, significativement plus élevée ($P < 0,05$) dans les cours d'eau à découvert que dans les cours d'eau à couvert forestier. On a noté une corrélation positive entre la production épilithique et la température de l'eau ($P < 0,05$). La production bactérienne hétérotrophe était supérieure à la production primaire nette dans les cours d'eau à couvert forestier, mais pas dans les cours d'eau à découvert.

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The river continuum concept suggests that the food base for consumers in forested headwater streams is mostly terrestrial carbon (Vannote et al. 1980; Minshall et al. 1985). The evidence for this is often derived from measurements of community autotrophy and community respiration (e.g. see Cummins 1974; Bott et al. 1985), commonly expressed as the *P/R* ratio. The relevance of this ratio to assess the food base of stream ecosystems has been under debate (Fisher and Likens 1973; Minshall 1978; Matson and Klotz 1983; Rosenfeld and Mackay 1987; Meyer 1989). As the debate continues, other

methods of assessing the food base of stream ecosystems, notably the microbial component, are being developed (e.g. see Findlay et al. 1984; Hudson et al. 1990). However, these methods have been applied only to a few lotic systems (e.g. Edwards and Meyer 1986; Findlay et al. 1986; Kaplan and Bott 1989; Stock and Ward 1989). The measurement of microbial heterotrophic production in streams is thus in its infancy, despite the generally believed importance of microorganisms to bioenergetic processes in stream ecology (e.g. Benke et al. 1988).

Recent studies indicate that heterotrophic bacterial production is indeed significant. In high-order blackwater rivers in Georgia, bacterial production in sediment exceeded primary production throughout most of the year (Findlay et al. 1986).

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According to Edwards and Meyer (1987), bacterioplankton carbon accounted for 20–67% of the daily black fly growth. In a second-order stream in Alabama, bacterial production in a riffle was 15–50% of primary production (Stock and Ward 1989).

No studies have concurrently examined the total bacterial contribution, i.e. from the water column, sediment, and epilithon in headwater streams. Heterotrophic bacterial productivity may play a significant part in community energetics of headwater streams. Therefore, we measured its magnitude in the water column, sediment, and epilithon of forested and open headwater streams and compared it with algal production. In addition, we examined its relationship to temperature, ash-free dry mass, and water column phosphorus. We examined the time course of incorporation of nucleoside, recovery efficiency of bacterial DNA, isotope dilution, and the possible introduction of disturbance artifacts in order to facilitate comparisons of bacterial production rates and to determine the appropriateness of the technique used to estimate bacterial production in these habitats (rate of incorporation of tritiated thymidine ($[^3\text{H}]\text{TdR}$) into bacterial DNA).

Materials and Methods

Study Sites

We sampled (under base flow) one site on each of two open streams (Swan Creek (SC) and Carol Creek (CC)) and three forested streams (Limestone Creek (LC), Hanlon Creek (HC), and a tributary to the West Credit River (WC)) in south-central Ontario between May and August 1988. Field experiments were performed between 12:30 and 17:30. The order of sampling of sites was randomized.

All sites were on second- or third-order stream sections (Table 1). Pool sediments at all sites consisted largely of fine to medium-size particles 100–500 μm in diameter. All riffles had an underlying cobble substratum. Riffle rocks at site LC were heavily coated with travertine. Further information concerning these streams is provided in Taylor and Roff (1982), Barton et al. (1985), Ashenden (1986), Bowlby and Roff (1986), and Hudson et al. (1990).

Water Column

Water column production was measured on one occasion at sites LC, WC, CC, and SC. Stream water (10–30 mL) was incubated for 15–30 min in acid-washed (0.1 N HCl) polypropylene tubes (50 mL). Cold thymidine (TdR, range 0–300 nM final concentration) and 1.48 MBq of $[^3\text{H}]\text{TdR}$ (13–47 nM final concentration) were injected into test tubes designated for isotope dilution (five treatments: three subsamples and one control per treatment) and production experiments (three subsamples

and one control). Isotope dilution experiments were conducted for each production experiment according to Pollard and Moriarty (1984). Experiments were stopped with an injection of NaOH (0.25 N final concentration) (Wicks and Roberts 1987). All samples (water column, sediment, and epilithic) were put on ice, transported to the laboratory, and stored at 5°C before being processed (within 18–24 h after stopping). Procedures for isolating DNA from water column samples were similar to those of Wicks and Roberts (1987). Additional water column samples were taken to determine total phosphorus (persulphate digestion; APHA 1980).

Tritiated DNA (thymine- $\text{Me-}^3\text{H}$) from *Escherichia coli* (New England Nuclear, 440 MBq $\cdot\text{mg}^{-1}$, cat. No. NET-561) was used to estimate recovery of bacterial DNA from all habitats during each production experiment. The above stock was diluted into a working solution which consisted of 25 μg of DNA from salmon testes (Sigma Chemical Co. cat. No. D-1626), 0.232 MBq of stock DNA in 5 mL of 10 mM Tris buffer, and 10 mM EDTA at pH 7 (procedure modified from Findlay et al. 1984). Two previously killed samples were injected with the $[^3\text{H}]\text{DNA}$ working solution, capped, and then placed alongside the production and isotope dilution test tubes.

Aliquots of the $[^3\text{H}]\text{DNA}$ working solution were radioassayed to determine the activity of the $[^3\text{H}]\text{DNA}$ added to samples in the field. The radioassay consisted of hydrolysing the aliquot of $[^3\text{H}]\text{DNA}$ in 1 mL of 10% (w/v) trichloroacetic acid (TCA) at 95°C for 1 h. The aliquot was counted as described in Hudson et al. (1990) and compared with the activity in recovery samples to determine the efficiency of bacterial DNA extraction.

Bacterial abundance and cell volume in the water column were estimated from two subsamples of stream water which was used for production and isotope dilution experiments. These subsamples were preserved (2% formaldehyde (v/v) final concentration), stained (DAPI), and filtered (25-mm Nuclepore polycarbonate filter, pore size 0.2 μm). Additional information on counting procedures can be found in Hudson et al. (1990).

Production in all habitats was calculated with the following general formula (modified from Findlay et al. 1984):

$$P = (\text{dpm in DNA}) / (\text{SA}) (\text{cells/TdR}) (\text{C/cell})$$

where dpm (disintegrations per minute) in DNA = dpm incorporated into DNA, SA (specific activity) = dpm of $[^3\text{H}]\text{TdR}$ added per effective pool concentration (determined from isotope dilution series), cells/TdR = cells produced per mole of TdR incorporated (2×10^{18} cells produced per mole of TdR incorporated; Moriarty 1986a), and C/cell = average carbon content per cell. A value of 1.2×10^{-13} g C $\cdot\mu\text{m}^{-3}$ (Watson et al. 1977) was applied (see Hudson et al. 1990 for further details).

TABLE 1. Description of the five study sites in southern Ontario.

	Hanlon Cr.	Limestone Cr.	West Credit R.	Carol Cr.	Swan Cr.
Site code	HC	LC	WC	CC	SC
Watershed	Speed R.	Bronte Cr.	Credit R.	Grand R.	Grand R.
Canopy type	Closed coniferous	Closed deciduous	Closed coniferous	Open meadow	Open meadow
Site latitude	43°30'20"	43°28'15"	43°47'55"	43°42'25"	43°39'25"
Site longitude	80°15'30"	79°56'00"	80°05'10"	80°32'15"	80°24'30"
Site elevation (m)	319	241	409	395	357
Stream order	2	2	2	2	3
Discharge ($\text{m}^3\cdot\text{s}^{-1}$) ^a	0.02	0.003	0.009	0.003	0.01

^aEach value was determined from one measurement taken during the field season with the velocity area technique using a Marsh–McBirney portable flow meter (model 201).

Sediment

Sediment and epilithic bacterial production was measured twice at each site. Four sediment cores (6.7 cm inside diameter) were taken from the pool sediments using a stratified random sampling design. The top 2 cm of sediment was diluted with an equal quantity of unsterilized stream water (production in water is negligible compared with sediment production, see below). The mixture was stirred to homogeneity and an aliquot (5 mL) was removed for production determination of each core. Additional samples were taken to determine sediment dry mass and ash-free dry mass (AFDM) of each core. AFDM was determined from the difference between the dry mass (55°C for 24 h) and the ash mass (500°C for 2 h) of samples.

Another sample (5 mL) of the above mixture was preserved (2% formaldehyde (v/v) final concentration) to determine cellular volume and abundance of bacteria in each core. In the laboratory, these samples were mixed to homogeneity and subsampled. They were then diluted with filter sterilized deionized water and homogenized (5 min in a Waring blender at approximately 21 000 rpm). An aliquot was removed, stained (DAPI), and filtered (25-mm Nuclepore polycarbonate filter, pore size 0.2 µm). One slide was prepared for each core. Detailed counting procedures can be found in Hudson et al. (1990).

The remainders of the top 2 cm of sediment from each core were combined together, stirred to homogeneity, and pipetted (5 mL) into an isotope dilution series. No pipetting biases in dry mass or AFDM were found across each isotope dilution series. TdR (0–3017 nM final concentration) and 1.48 MBq of [³H]TdR (95–148 nM final concentration) were injected into the isotope dilution and production test tubes. Samples were periodically shaken during incubation (15–31 min) at ambient stream temperature. Incubations were stopped with 0.6 N NaOH, 0.1% sodium dodecyl sulphate (SDS), and 25 mM EDTA (final concentrations) (Bell and Ahlgren 1987).

In the laboratory, sediment samples were placed in a water bath at 95°C for 1 h (Bell and Ahlgren 1987), cooled, and centrifuged (350 × g). An aliquot of the supernatant was extracted, chilled, and neutralized (HCl). The chilled aliquot was acidified with TCA (5% (w/v) final concentration). The DNA in these aliquots was then isolated as described in Hudson et al. (1990). Bacterial production in sediments was determined from the general formula above.

The time course of incorporation of [³H]TdR into sediment DNA was examined at one of the more productive sites (SC). Field and laboratory procedures for the time course experiment were similar to those of the production and isotope dilution experiments. The top 2 cm of four sediment cores were combined together and diluted with an equal quantity of stream water. This mixture was stirred and pipetted (5 mL) into eight time treatments where each treatment consisted of a previously killed control and two live samples. Incubations were started with an injection of [³H]TdR (1.48 MBq, 114 nM final concentration) and were stopped after 5, 10, 15, 20, 31, 40, 60, or 90 min. Incubation temperature was kept constant and near ambient stream temperature.

Although the technique of measuring bacterial production via the rate of incorporation of [³H]TdR into DNA is less subject to disturbance artifacts (Findlay et al. 1984) than other techniques, Moriarty (1986a) stated that further work is needed to determine the responses of DNA synthesis in sediments to handling. Our method of mixing the sediment could introduce a considerable disturbance artifact. Therefore, an experiment was conducted at site SC to examine this possibility. Field and laboratory methods were similar to those of the production and

isotope dilution experiments. The top 2 cm of four sediment cores were combined and diluted with an equal quantity of stream water. Aliquots (5 mL) of stirred sediment were pipetted into test tubes of six treatments. Each treatment consisted of one control and two experimental samples. The injection of [³H]TdR was staggered in time from the first to the last treatment. All treatments were incubated for 15 min. For example, treatment 1 was injected at 0 min with [³H]TdR and was stopped at 15 min, while treatment 6 was injected at 90 min and stopped at 105 min into the experiment. If the uptake of [³H]TdR into DNA was not equal among treatments, then a disturbance artifact was probably introduced. Incubation temperatures were kept constant and near ambient stream temperature.

Epilithon

Epilithic methods are fully described in Hudson et al. (1990); however, additional experiments were conducted to simplify the technique. In these experiments, we questioned the correlation between the recovery of added [³H]DNA and the area of epilithic growth on rock chips (p. 1816, fig. 2 in Hudson et al. 1990), which we believed to be spurious. If the relationship is spurious, then variation in the epilithic surface area on rock chips should not affect efficiency of recovery of [³H]DNA.

In the fall of 1990, a rock from site HC (a forested stream) was carefully removed from the riffle and transported at ambient stream temperature to the laboratory. The rock, with attached epilithon, was broken into chips. Ten rock chips were randomly selected and cracked in half. One half was placed in a test tube containing 8 mL of stream water. This experimental sample was killed (0.6 N NaOH, 0.1% SDS, and 25 mM EDTA final concentration) and injected with an aliquot (6.5 kBq) of the tritiated DNA working solution (described in the water column methods). Experimental samples were then capped and rotated in a water bath (30 min at 15 rpm) and processed as recovery samples according to Hudson et al. (1990). Two controls (no rock chip) and two background (no [³H]DNA) samples accompanied experimental samples. The order in which experimental samples were processed (e.g. filtering) was randomized.

The second half from each rock chip was used to determine the AFDM on adjacent chips used in the above experiment. AFDM and surface area of the epilithon on rock chips were determined as described in Hudson et al. (1990). Epilithic surface area and AFDM were regressed against recovery efficiency for each experimental chip. The above experiment was repeated for a second rock which was removed from site SC (a meadow stream).

Statistical Analyses

Water column production data were log transformed and tested for significant differences (*t*-test, *n* = 4) between forested and open sites. Mean sediment production and doubling time values (*n* = 10) were also log transformed and tested for significant differences between forested and open sites using analysis of variance (ANOVA). Analysis of covariance (ANCOVA) was used to assess the influence of temperature, AFDM, and total phosphorus on production rate and turnover time. Relationships between production and turnover and the environmental variables, regardless of treatment (forested or open), were assessed using linear regression. Mean epilithic production and doubling time values (*n* = 10) were treated in a manner similar to sediment data. All results are expressed as mean ± 95% CI, unless stated otherwise.

Results

Water Column

Technique

Mean recovery of added [^3H]DNA was $59 \pm 26\%$ from water column samples. Effective pool concentrations diluting added TdR had a range of 15–65 nM and were greatest in open streams (Table 2). The replicates of the fifth treatment (highest concentration of added TdR) of water column and sediment isotope dilution series from open streams lay below the regression line (e.g. see Fig. 1). These replicates were not included in the calculation of isotope dilution.

Ecology

Water column temperatures were consistently warmer in open streams (Table 2). Total phosphorus concentrations ranged from 4.2 to $30.8 \mu\text{g}\cdot\text{L}^{-1}$ and did not differ significantly between forested and open streams (t -test, $P > 0.05$, $n = 10$). Bacterial cell volume, abundance, and biomass in open streams exceeded those in forested streams (Table 2). Abundance and biomass were significantly different in the two systems (t -test, $P < 0.05$).

Bacterial productivity was at least an order of magnitude greater in open streams than in forested streams (Table 2). Meanwhile, bacterial doubling times in open streams were a tenth of those in forested streams. However, these differences in production rate and doubling time between open and forested streams were not significant (using $\log_{10}$ -transformed data, t -test, $P > 0.05$).

Sediment

Technique

The mean dry mass of sediment used in the production incubations was 0.47 ± 0.20 g at forested streams and 1.5 ± 1.0 g at open streams (Tables 3 and 4). Open streams had coarser sediments.

Mean recovery efficiency of [^3H]DNA from the sediment ($63 \pm 9.4\%$) was greatest for open streams (Tables 3 and 4); recovery efficiency was not related ($P > 0.05$) to sediment AFDM.

Effective pool concentrations diluting added TdR in the sediments had a range of 226–7103 nM (Tables 3 and 4). These

concentrations are adjusted for the twofold dilution of the sediment with stream water in the field (see Materials and Methods).

The time course of [^3H]TdR incorporation into DNA (Fig. 2) was linear from 5 to 20 min ($r^2 = 0.94$, $P < 0.05$). A disturbance artifact was detected (Fig. 3) at site SC: incorporation of [^3H]TdR increased per unit time during the period between the addition of the sediment to all treatments (time = 0) and the termination of the last treatment (time = 105 min) ($r^2 = 0.90$, $P < 0.05$, slope = 370 ± 88 dpm in DNA $\cdot\text{min}^{-1}\cdot\text{g dry mass}^{-1}$).

Ecology

In most cases, sediment incubations were conducted at lower temperatures in forested streams than in open streams (Tables 3 and 4). The mean AFDM in the top 2 cm of sediments of forested streams was $1440 \pm 284 \text{ g}\cdot\text{m}^{-2}$, while in sediments of open streams it was only $853 \pm 546 \text{ g}\cdot\text{m}^{-2}$. Mean contribution of AFDM to sediment dry mass was $46 \pm 12\%$ in forested streams and $7.8 \pm 2.2\%$ in open streams.

Mean bacterial cell volume in sediments of forested ($0.20 \pm 0.042 \mu\text{m}^3$) and open streams ($0.18 \pm 0.073 \mu\text{m}^3$) was similar (Tables 3 and 4). However, bacterial abundance ($4.1 \times 10^{13} \pm 1.2 \times 10^{13} \text{ cells}\cdot\text{m}^{-2}$) and biomass ($0.95 \pm 0.25 \text{ g C}\cdot\text{m}^{-2}$) in sediments of forested streams were greater than those in sediments of open streams ($2.7 \times 10^{13} \pm 2.0 \times 10^{13} \text{ cells}\cdot\text{m}^{-2}$ and $0.56 \pm 0.40 \text{ g C}\cdot\text{m}^{-2}$, respectively).

Mean bacterial production in forested stream sediments ($70 \pm 38 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) was not significantly different (ANOVA, $P > 0.05$) from that in sediments of open streams ($93 \pm 55 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$). There were no significant linear relationships ($P > 0.05$) between production in the sediment and temperature, mean AFDM, or water column phosphorus. Doubling times had a range of 6.4–63 h in sediments of forested streams and 2.8–17 h in sediments of open streams (Tables 3 and 4). These times were not significantly different ($P > 0.05$), nor were they related ($P > 0.05$) to the other variables examined.

Epilithon

Technique

The [^3H]DNA added was recovered with a mean efficiency of $58 \pm 11\%$ from epilithic samples (Tables 5 and 6). In initial experiments, recovery efficiency appeared to be positively

TABLE 2. Bacterial production rates and associated variables in the water column at each research site.

	Forested sites		Open sites	
	Limestone Cr.	West Credit R.	Carol Cr.	Swan Cr.
Sampling data ^a	16/05/88	28/07/88	31/05/88	10/08/88
Water temperature ($^{\circ}\text{C}$)	17	17.5	29	27.5
Cell volume (μm^3) ^b	0.17	0.097	0.24	0.22
Cell abundance ($\text{cells}\cdot\text{L}^{-1} \times 10^9$) ^b	0.91	1.0	3.4	3.0
Cell biomass ($\mu\text{g C}\cdot\text{L}^{-1}$) ^{bc}	18	12	97	80
DNA recovery (%)	54	65	78	39
Incubation length (min)	30	20	34	15
Effective pool concn. (nM) ^d	24 (0.91)	15 (0.93)	65 (0.93)	37 (0.66)
Production ($\mu\text{g C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$) ^b	0.54	0.21	13	67
Doubling time (h) ^{be}	34	57	7.8	1.2

^aDate is listed as day/month/year.

^bMean of two or three subsamples.

^cBiomass = cell abundance ($\text{cells}\cdot\text{L}^{-1}$) $\times$ carbon per cell ($1.2 \times 10^{-13} \text{ g C}\cdot\mu\text{m}^{-3}$).

^dObtained from linear isotope dilution plots. Followed (in parentheses) with the coefficient of determination (r^2).

^eEqual to biomass divided by production.

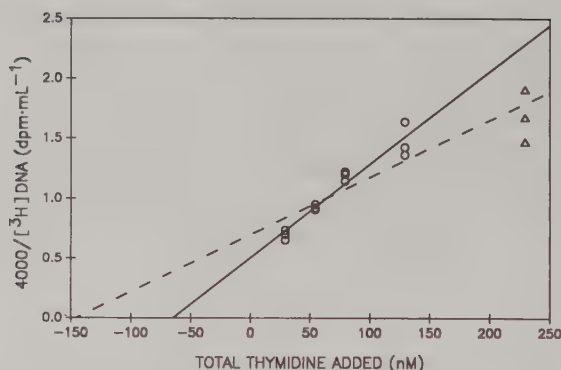


FIG. 1. Example of a nonlinear isotope dilution series (from the water column of Carol Creek on May 31). The positive x-axis is the concentration of TdR added (labelled and nonlabelled). The y-axis is the reciprocal of radioactivity (dpm in DNA · mL stream water). The intercept on the negative x-axis is the effective pool concentration that dilutes the added TdR. Replicates are corrected for control counts. Note the difference in isotope dilution when the fifth treatment replicates (Δ) are included in the regression (broken line) and when they are not (solid line). The latter regression is used to calculate isotope dilution when the fifth treatment departs from linearity (see text for further information).

related to the mean epilithic surface area of rock chips ($r^2 = 0.52$, $P < 0.05$, $n = 10$). When we performed additional experiments (outlined in Materials and methods) to test the validity of this relationship, we discovered that it was spurious ($P > 0.05$). In these additional experiments, recovery efficiency was still not significantly related ($P > 0.05$) to AFDM.

The effective pool concentrations diluting added TdR had a range of 63–440 nM (Tables 5 and 6). The greatest concentration (from site LC on June 28) is probably an overestimate, since it was derived from an isotope dilution plot with a low coefficient of determination ($r^2 = 0.16$). Further evaluation of epilithic methodology is presented in Hudson et al. (1990).

Ecology

Epilithic production and isotope dilution experiments were conducted at lower ambient water temperatures in forested streams than in open streams (Tables 5 and 6). Mean AFDM on rocks chosen for production measurements was similar in forested ($9.9 \pm 7.1 \text{ mg C} \cdot \text{cm}^{-2}$) and open streams ($11.8 \pm 7.0 \text{ mg C} \cdot \text{cm}^{-2}$). The variation about the mean AFDM within a site (Tables 5 and 6) was high and indicative of the heterogeneity of the epilithon.

Epilithic AFDM at site LC was very high (11 and $23 \text{ mg} \cdot \text{cm}^{-2}$) (Tables 5 and 6). The rock surfaces in this stream were characterized by a thick layer of travertine (up to 1 cm). We discovered that approximately 90% of the AFDM was in the travertine layer and not in the epilithon. Therefore the AFDM for this site is overestimated.

Bacterial cell volume in the epilithon of forested streams (range $0.15\text{--}0.25 \mu\text{m}^3$) was similar to that of open streams (range $0.17\text{--}0.31 \mu\text{m}^3$) (Tables 5 and 6). Mean bacterial abundance in the epilithon of forested streams ($2.5 \times 10^{12} \pm 1.3 \times 10^{12} \text{ cells} \cdot \text{m}^{-2}$) was also similar to that in open streams ($2.9 \times 10^{12} \pm 2.0 \times 10^{12} \text{ cells} \cdot \text{m}^{-2}$). Mean bacterial biomass had a range of 35–145 $\text{mg C} \cdot \text{m}^{-2}$ and was similar at both types of sites.

Mean epilithic production in forested streams ($2.9 \pm 1.1 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) was significantly lower (ANOVA, $P < 0.05$) than that in open streams ($28 \pm 32 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) (Tables 5 and 6). Mean bacterial production was positively related ($r^2 = 0.50$, $P < 0.05$) to stream water temperature (Fig. 4). Differences in production between forested and open streams were not significant (ANCOVA, $P > 0.05$) when the effect of temperature was removed.

Mean bacterial doubling times (Tables 5 and 6) in forested streams were significantly greater than those in open streams (ANOVA, $P < 0.05$). Doubling times were negatively related to stream water temperature ($r^2 = 0.45$, $P < 0.05$) and unrelated to AFDM and total phosphorus in the water column. Doubling times were not significantly different between forested and open streams when differences in temperature were statistically controlled (ANCOVA, $P > 0.05$).

TABLE 3. Bacterial production rates and associated variables in the upper 2 cm of sediment on the first sampling date at each research site.

	Forested sites			Open sites	
	Hanlon Cr.	Limestone Cr.	West Credit R.	Carol Cr.	Swan Cr.
Sampling date ^a	09/06/88	16/06/88	12/07/88	07/06/88	05/07/88
Water temperature ($^{\circ}\text{C}$)	9.3	17	16	19.5	29
Mean dry mass incubated (g^b)	0.33 ± 0.063 (4)	0.39 ± 0.047 (4)	0.83 ± 1.3 (4)	1.5 ± 1.3 (4)	1.4 ± 0.58 (5)
Mean AFDM ($\text{g} \cdot \text{m}^{-2}$) ^b	1470 ± 203 (4)	1310 ± 102 (4)	1050 ± 509 (4)	626 ± 110 (4)	818 ± 101 (5)
Mean cell volume (μm^3) ^{bc}	0.24 ± 0.039 (4)	0.25 ± 0.086 (4)	0.17 ± 0.077 (4)	0.24 ± 0.16 (4)	0.16 ± 0.053 (5)
Mean cell abundance ($\text{cells} \cdot \text{m}^{-2} \times 10^{13}$) ^{bc}	3.9 ± 0.91 (4)	3.0 ± 1.6 (4)	5.4 ± 1.8 (4)	1.4 ± 0.36 (4)	3.7 ± 0.51 (5)
Mean cell biomass ($\text{g C} \cdot \text{m}^{-2}$) ^{bcd}	1.1 ± 0.25 (4)	0.90 ± 0.42 (4)	1.1 ± 0.51 (4)	0.41 ± 0.34 (4)	0.72 ± 0.22 (5)
DNA recovery (%)	51	50	64	66	68
Incubation length (min)	30	31	20	30	16
Effective pool concn. (nM) ^e	226 (0.87)	2208 (0.97)	2747 (0.89)	4062 (0.95)	2740 (0.89)
Mean production ($\text{mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) ^b	18 ± 1.7 (4)	78 ± 38 (4)	73 ± 35 (4)	140 ± 87 (4)	98 ± 14 (5)
Mean doubling time (h) ^{bf}	63 ± 11 (4)	13 ± 12 (4)	15 ± 5.2 (4)	2.8 ± 1.2 (4)	7.5 ± 2.7 (5)

^aDate is listed as day/month/year.

^bListed as mean, 95% CI, and sample size (four to five replicates per site visit). Dry mass and AFDM are for production samples.

^cSample size is equal to the number of independent, replicate slides counted.

^dBiomass = cell abundance ($\text{cells} \cdot \text{m}^{-2}$) $\times$ carbon per cell ($1.2 \times 10^{-13} \text{ g C} \cdot \mu\text{m}^{-3}$).

^eObtained from linear isotope dilution plots. Followed (in parentheses) with the coefficient of determination (r^2).

^fEqual to biomass divided by production.

TABLE 4. Bacterial production rates and associated variables in the upper 2 cm of sediment on the second sampling date at each research site.

	Forested sites			Open sites	
	Hanlon Cr.	Limestone Cr.	West Credit R.	Carol Cr.	Swan Cr.
Sampling date ^a	19/07/88	02/08/88	28/07/88	15/07/88	04/08/88
Water temperature (°C)	15	21	17	26	29.3
Mean dry mass incubated (g) ^b	0.34 ± 0.091 (4)	0.49 ± 0.023 (4)	0.43 ± 0.11 (4)	2.4 ± 1.4 (4)	0.82 ± 0.32 (4)
Mean AFDM (g·m ⁻²) ^b	1590 ± 298 (4)	1850 ± 197 (4)	1370 ± 242 (3)	1350 ± 342 (4)	618 ± 208 (4)
Mean cell volume (μm ³) ^{bc}	0.16 ± 0.063 (4)	0.19 ± 0.048 (4)	0.17 ± 0.075 (4)	0.13 ± 0.08 (4)	0.17 ± 0.080 (4)
Mean cell abundance (cells·m ⁻² × 10 ¹³) ^{bc}	2.9 ± 0.48 (4)	3.7 ± 1.6 (4)	5.7 ± 2.0 (4)	1.9 ± 1.7 (4)	3.8 ± 2.0 (4)
Mean cell biomass (g C·m ⁻²) ^{bcd}	0.54 ± 0.19 (4)	0.85 ± 0.40 (4)	1.2 ± 0.88 (4)	0.30 ± 0.21 (4)	0.82 ± 0.83 (4)
DNA recovery (%)	43	71	62	62	90
Incubation length (min)	30	30	30	20	15
Effective pool concn. (nM) ^e	1760 (0.84)	7103 (0.84)	1792 (0.97)	3340 (0.87)	1827 (0.95)
Production (mg C·m ⁻² ·h ⁻¹) ^b	93 ± 61 (4)	120 ± 35 (4)	40 ± 22 (4)	70 ± 48 (4)	64 ± 69 (4)
Doubling time (h) ^{bf}	6.4 ± 3.5 (4)	7.0 ± 2.4 (4)	29 ± 12 (4)	4.2 ± 0.54 (4)	17 ± 19 (4)

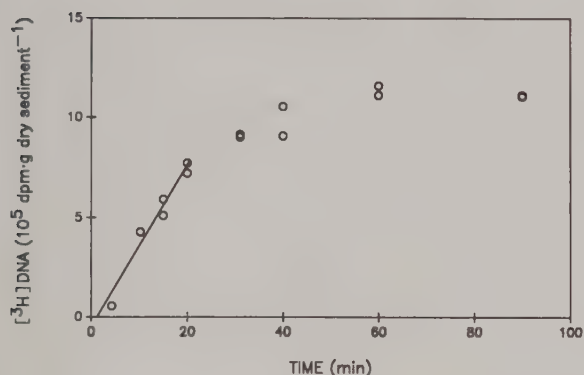
^aDate is listed as day/month/year.^bListed as mean, 95% CI, and sample size (three to four replicates per site visit). Dry mass and AFDM are for production samples.^cSample size is equal to the number of independent, replicate slides counted.^dBiomass = cell abundance (cells·m⁻²) × carbon per cell (1.2 × 10⁻¹³ g C·μm⁻³).^eObtained from linear isotope dilution plots. Followed (in parentheses) with the coefficient of determination (*r*²).^fEqual to biomass divided by production (four replicates per site visit).

FIG. 2. Time course of [³H]TdR incorporation into bacterial DNA at Swan Creek (July 5) at 29°C using a mean dry mass of 1.5 ± 0.19 g (*n* = 4) of sediment per sample. The y-axis is dpm in bacterial DNA·g dry sediment⁻¹ (corrected for control counts). The x-axis is the length of time a sample was incubated with [³H]TdR (1.48 MBq·sample⁻¹, 114 nM final concentration). Incorporation for the first 20 min of incubation is linear (*r*² = 0.94, *n* = 7, y-axis intercept = -56 200 ± 163 000).

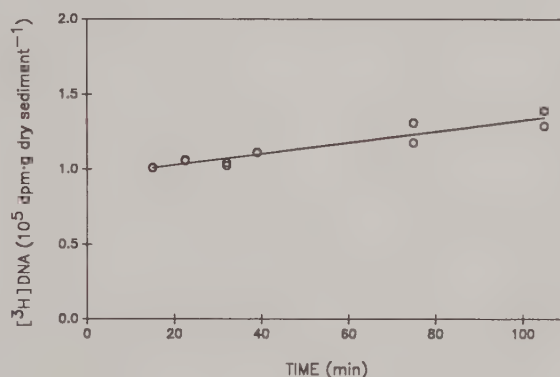


FIG. 3. Detection of a disturbance artifact (dpm·g dry sediment⁻¹ = 370.3 (min) + 95 475, *r*² = 0.90, *P* < 0.05, *n* = 12) in the sediment at Swan Creek (August 16) at 26°C with a mean sediment sample dry mass of 0.95 ± 0.15 g (*n* = 4). The y-axis is dpm in bacterial DNA·g dry sediment⁻¹ (corrected for control counts). Sediment was cored 16 min before it was added to all the test tubes. The x-axis is the total length of time (up to 105 min) that sediment sat in test tubes of each treatment. Treatments consist of two replicates and one control. The addition of sediment to all test tubes is equal to 0 min on the x-axis. The injection of [³H]TdR into each treatment was staggered in time from the first to the last treatment. All treatments were incubated for 15 min. For example, treatment 1 was injected at 0 min with [³H]TdR and was stopped at 15 min, while treatment 6 was injected at 90 min and stopped at 105 min into the experiment.

Discussion

Water Column

Technique

The range of effective pool concentrations (Table 2) diluting added TdR is substantial, particularly in open streams (37–65 nM). In order to inhibit the *de novo* pathway, and thus avoid conducting isotope dilution experiments (see Pollard and Moriarty 1984; Bell 1986), concentrations of TdR greater than 10 nM may have to be added.

Sediment and water column isotope dilution series from open streams are not linear when the fifth treatments are included in the regressions (e.g. Fig. 1). This nonlinearity at the highest concentrations of added TdR has been observed by others

(Pollard and Moriarty 1984; Riemann and Sondergaard 1984; Bell 1986; Hollibaugh 1988) and is often referred to as a “right shift”. The high concentrations of TdR added to the fifth treatments may have been sufficient to inhibit or decrease further contributions of thymidine triphosphate from the *de novo* pathway (see Pollard and Moriarty 1984; Bell 1986). This would result in an increased use of the added TdR and a disproportionately high labelling of DNA with [³H]TdR by cells in the fifth treatment replicates.

TABLE 5. Bacterial production rates and associated variables in the epilithon on the first sampling date at each research site.

	Forested sites			Open sites	
	Hanlon Cr.	Limestone Cr.	West Credit R.	Carol Cr.	Swan Cr.
Sampling date ^a	21/06/88	28/06/88	12/07/88	03/06/88	30/06/88
Water temperature (°C)	13	16	16	20	20
Mean AFDM (mg·cm ⁻²) ^b	3.6 ± 4.5 (4)	11 ± 4.9 (4)	6.9 ± 6.8 (3)	8.3 ± 6.7 (4)	18 ± 36 (4)
Mean cell volume (μm ³) ^{bc}	0.32 ± 0.12 (4)	0.27 ± 0.16 (4)	0.23 ± 0.073 (4)	0.30 ± 0.17 (4)	0.17 ± 0.046 (4)
Mean cell abundance (cells·m ⁻² × 10 ¹²) ^{bc}	1.1 ± 0.41 (4)	3.7 ± 0.32 (4)	2.5 ± 0.66 (4)	4.3 ± 1.4 (4)	3.5 ± 0.98 (4)
Mean cell biomass (g C·m ⁻²) ^{bcd}	41 ± 12 (4)	120 ± 76 (4)	70 ± 35 (4)	150 ± 38 (4)	70 ± 7.0 (4)
DNA recovery (%)	76	41	68	66	46
Incubation length (min)	60	90	45	30	20
Effective pool concn. (nM) ^e	85 (0.66)	440 (0.16)	63 (0.37)	209 (0.50)	267 (0.60)
Mean production (mg C·m ⁻² ·h ⁻¹) ^b	2.5 ± 1.7 (4)	4.6 ± 3.9 (4)	3.0 ± 6.9 (3)	37 ± 70 (4)	8.2 ± 6.3 (4)
Mean doubling time (h) ^{bf}	20 ± 19 (4)	27 ± 7.9 (4)	49 ± 115 (3)	7.6 ± 7.4 (4)	11 ± 8.9 (4)

^aDate is listed as day/month/year.^bListed as mean, 95% CI, and sample size (three to four replicates per site visit). Epilithic AFDM is for production samples.^cSample size is equal to the number of independent, replicate slides counted.^dBiomass = cell abundance (cells·m⁻²) × carbon per cell (1.2 × 10⁻¹³ g C·μm⁻³).^eObtained from linear isotope dilution plots. Followed (in parentheses) with the coefficient of determination (*r*²).^fEqual to biomass divided by production.

TABLE 6. Bacterial production rates and associated variables in the epilithon on the second sampling date at each research site.

	Forested sites			Open sites	
	Hanlon Cr.	Limestone Cr.	West Credit R.	Carol Cr.	Swan Cr.
Sampling date ^a	19/07/88	02/08/88	28/07/88	15/07/88	04/08/88
Water temperature (°C)	15.3	21	17.5	26.3	30
Mean AFDM (mg·cm ⁻²) ^b	8.7 ± 4.9 (4)	23 ± 20 (4)	6.0 ± 5.4 (4)	9.7 ± 7.8 (4)	11 ± 3.2 (4)
Mean cell volume (μm ³) ^{bc}	0.25 ± 0.051 (4)	0.19 ± 0.082 (4)	0.15 ± 0.11 (4)	0.31 ± 0.17 (4)	0.30 ± 0.10 (4)
Mean cell abundance (cells·m ⁻² × 10 ¹²) ^{bc}	1.4 ± 1.4 (4)	4.2 ± 4.6 (4)	2.1 ± 0.96 (4)	1.4 ± 1.7 (4)	2.3 ± 1.5 (4)
Mean cell biomass (g C·m ⁻²) ^{bcd}	42 ± 47 (4)	84 ± 62 (4)	35 ± 23 (4)	46 ± 51 (4)	84 ± 69 (4)
DNA recovery (%)	36	46	56	73	78
Incubation length (min)	76	60	30	20	20
Effective pool concn. (nM) ^e	93 (0.67)	107 (0.64)	179 (0.44)	196 (0.87)	272 (0.78)
Mean production (mg C·m ⁻² ·h ⁻¹) ^b	2.6 ± 1.6 (4)	1.3 ± 1.2 (4)	3.2 ± 4.1 (4)	15 ± 24 (4)	51 ± 52 (4)
Mean doubling time (h) ^{bf}	18 ± 23 (4)	105 ± 171 (4)	15 ± 13 (4)	4.1 ± 4.0 (4)	2.0 ± 1.8 (4)

^aDate is listed as day/month/year.^bListed as mean, 95% CI, and sample size (four replicates per site visit). Epilithic AFDM is for production samples.^cSample size is equal to the number of independent, replicate slides counted.^dBiomass = cell abundance (cells·m⁻²) × carbon per cell (1.2 × 10⁻¹³ g C·μm⁻³).^eObtained from linear isotope dilution plots. Followed (in parentheses) with the coefficient of determination (*r*²).^fEqual to biomass divided by production.

Ecology

Mean bacterial volume, abundance, and biomass in the water column of forested streams (Table 2) are similar to other reported values for springs and forested streams (Rheinheimer 1985; Palumbo et al. 1987). However, values of these in open streams more closely approximate values of small rivers (Rheinheimer 1985; Edwards and Meyer 1986; Edwards 1987; Servais 1989). This difference may be related to the different nutritional conditions or trophic status of forested and open streams.

Bacterioplankton production (Table 2) was probably near its annual maximum because measurements were taken at peak daytime temperatures and presumably at peak water column dissolved organic carbon (DOC) concentrations (e.g. Kaplan and Bott 1982, 1989; Sondergaard and Jensen 1986; Robarts 1988). Production rates in forested streams were similar to those in two Georgian blackwater rivers (Edwards and Meyer 1986) and to those in a Swedish lake (Bell et al. 1983). Production in open streams was similar to estimates from the Meuse River

(Servais 1989) and from aquaculture ponds (Moriarty 1986b). Mean doubling times of bacteria (1.2–57 h) overlapped with those in the studies cited above.

Production rates in open streams were 10–100 times greater than those in forested streams. However, with only two samples per treatment and considerable variation within each treatment, a statistically significant difference was not demonstrated.

Sediment

Technique

The recovery efficiency of [³H]DNA and the effective pool concentrations diluting added TdR fluctuated over relatively small temporal and spatial scales within each stream habitat (Tables 2–6). Therefore, we could not assume that they would remain constant between sites or within a site between sampling dates. Thus, recovery and isotope dilution experiments were performed with each production experiment.

The effective pool concentrations in the sediment that diluted the added TdR exceeded the effective pool concentrations

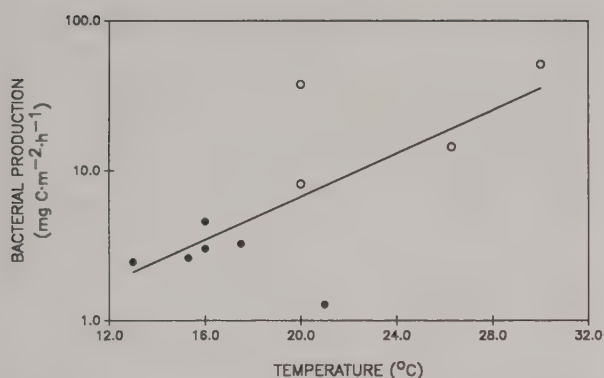


FIG. 4. Relationship between water temperature and mean epilithic bacterial production at each site throughout the sampling season ($\log_{10} (\text{mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}) = 0.0721 (^\circ\text{C}) - 0.616$, $r^2 = 0.50$, $P < 0.05$, $n = 10$). Each point is a mean of four replicates. Estimates from forested (●) and open (○) sites are shown.

measured in the epilithon and water column by one to two orders of magnitude. These concentrations in the sediment are similar to those reported in other lotic sediments (see Table 7 and Findlay et al. 1984).

The time course of [^3H]TdR incorporation into bacterial DNA at site SC (Fig. 2) departed from linearity after 20 min of incubation at 29°C (mean dry mass of samples was 1.5 ± 0.19 g). Sediments were occasionally incubated for 30 min and may therefore have experienced substrate limitation. However, most of the incubations were done either at lower temperatures

(Tables 3 and 4) or with less sediment per replicate, or both, than in the time course experiment. Therefore, while substrate limitation may have occurred, it may not have significantly influenced incorporation rates.

The disturbance artifact experiment (Fig. 3) indicated a 6% overestimate of dpm in DNA·g dry mass of sediment $^{-1}$ in production and isotope dilution estimates from site SC. However, because conditions vary between sites and even among experiments at a site, this overestimate should only be viewed as an indication of the magnitude of the artifact introduced. In general, we assume that the production estimates were not seriously affected by this type of disturbance.

It should be recalled that this experiment measured the change in the rate of [^3H]TdR incorporation into DNA after the sediments were mixed to homogeneity. The experiment might have been more informative if it had been applied before the sediment had been mixed. However, as indicated by Moriarty and Pollard (1981), sediments often have to be mixed before dispensing; otherwise, sample heterogeneity may be excessive. Furthermore, this initial disturbance probably has little effect on [^3H]TdR incorporation into DNA as shown by Dobbs et al. (1989).

Ecology

Estimates of bacterial abundance and biomass in the stream sediments are similar to those reported from other sediment habitats (Table 7). The bacterial cell volumes are large, but similar to those of mangrove sediments (Alongi 1988) and salt marsh sediments (Ruble 1982).

Our production estimates pertain to the bacteria in the top 2 cm of oxygenated sediment. Anoxic sediments were typically

TABLE 7. Comparison of bacterial production rates and associated variables from aquatic sediments (NA = not available).

Source and habitat	Range or mean cell abundance (cells·m $^{-2}$ × 10 13) ^a	Range or mean cell volume (μm 3) and biomass (g C·m $^{-2}$) ^a	Effective pool concn. (nM)	Range or mean production ^b (mg C·m $^{-2}$ ·h $^{-1}$) ^a	Range or mean doubling time (d)
This study, top 2 cm of forested stream sediments	2.9–5.7 ^c (or 9.3–17) ^d	0.16–0.25 ^c 0.54–1.2 ^c (or 0.21–0.43) ^e	226–7103	18–120 ^c (or 6.8–37) ^f	0.27–2.6 ^c
This study, top 2 cm of open canopy stream sediments	1.4–3.8 ^c (or 1.1–6.2) ^d	0.13–0.24 ^c 0.30–0.82 ^c (or 0.018–0.14) ^e	1792–4062	64–140 ^c (or 4.2–13) ^f	0.12–0.71 ^c
Austin and Findlay 1989, top 1 cm of estuarine sediments	NA	NA 0.38–1.29	NA	0.08–9.4	4–687
Findlay et al. 1986, top 1 cm of river sediments	0.2–50 ^d	0.11 0.003–1.5	NA	0.01–22	Up to approx. 130 d
Meyer 1988, top 1 cm of river sediments	0.55	0.091 0.11	100–20 000	2.1	5.7
Bell and Ahlgren 1987, top 2 cm of lake sediments	30–150 ^d	0.157 5.7–20 ^c	NA	11.6–27.9 ^f	2.3–250
Alongi 1988, top 2 cm of mangrove sediments	2–360 ^d	0.15–0.35 NA	NA	8.3–213	0.2–5.5
Moriarty 1986b, top 1 cm of pond sediments	NA	NA 1.4–6.4	NA	4.6–22	4–10

^aIn these units unless listed otherwise.

^bCalculated via the rate of incorporation of tritiated thymidine.

^cRange of mean values of each site.

^dIn cells·g dry sediment $^{-1}$ × 10 9 .

^eIn mg C·g dry sediment $^{-1}$.

^fIn μg C·g dry sediment $^{-1}$.

encountered below this depth. Although these data may be considered as biased low, in terms of total (aerobic and anaerobic) bacterial production, they are unlikely to be grossly underestimated because production under anoxic conditions is much reduced (Moriarty 1986a).

Bacterial production rates in the sediments may be maximal because production was measured in the afternoon, when ambient temperatures would be at their highest. Bacterial production has been reported to be directly related to ambient temperature (e.g. see Kaplan and Bott 1989).

Production estimates reported for sediments of a eutrophic Swedish lake and for mangrove sediments are similar to our values for headwater stream sediments (Table 7). On the other hand, river, estuary, and pond sediments appear to be less productive (Table 7). These riverine estimates may be less than ours because of the sediment particle size differences between the studies: Austin and Findlay (1989) have found that bacteria in coarser sediments appear to be less productive than those in finer sediments.

Findlay et al. (1986) found that bacterial production was directly related to sediment organic content, while Moriarty and Pollard (1981) and Bell and Ahlgren (1987) found that bacterial production was directly related to changes in seasonal temperatures. In our headwater stream sediments, AFDM and temperature were not significantly related to production. However, sediment AFDM was lower in open streams than in forested streams, and temperature was higher in open streams than in forested streams. The influence of one parameter may thus negate the effect of the other, which may explain the lack of significant differences in production rates between forested and open streams and the lack of significant relationships between production and the independent variables, e.g. AFDM. However, the majority of our production estimates fall within the 90% CI of the general relationship between organic content and benthic production proposed by Cole et al. (1988).

Doubling times were quite short (Tables 3 and 4) at all stream sites, but are similar to those in mangrove sediments (Alongi 1988) (Table 7) and other stream sediments (Bott and Kaplan 1985).

Epilithon

Technique

Although the recovery efficiency of [^3H]DNA was reported to be significantly related to epilithic chip surface area by Hudson et al. (1990), we suspected that this relationship was spurious; our more recent and extensive experiments have not verified it. We now believe that epilithic chips do not have to be of identical surface area to avoid a bias in recovery efficiency.

Isotope dilution of added TdR is intermediate to that measured in the water column and sediment habitats. Dilution of [^3H]TdR was greater in open streams than in forested streams (with the exception of site LC which is probably biased, see Results). This may be explained by the greater quantity of living matter (i.e. periphyton) in open streams. As in the sediments, isotope dilution was often different at the same site on different dates. Further description of epilithic techniques (e.g. recovery and disturbance artifacts) is presented in Hudson et al. (1990).

Ecology

Mean bacterial volume ($0.25 \mu\text{m}^3$) in the epilithon was greater than in the water column or sediment habitats. However, epilithic bacterial abundance and biomass were one to two orders of magnitude less than in the sediment habitat (2 cm depth). The abundances of epilithic bacteria are similar to those

reported in other streams (Haack and McFeters 1982a; Goulder 1988).

Mean bacterial biomass is only $1.1 \times 10^{-4}\%$ of the mean AFDM on rock surfaces (data from site LC not included in the means, see Results). In pool sediments, mean bacterial biomass represents a greater proportion of the mean AFDM ($7.3 \times 10^{-2}\%$). Thus, the increased surface area of sediment particles compared with rock surfaces appears to support a larger biomass to AFDM ratio.

Diel and seasonal cycles in epilithic bacterial activity have been documented in streams (e.g. Haack and McFeters 1982b; Sinsabaugh and Linkins 1988; Kaplan and Bott 1989). For example, Kaplan and Bott (1989) measured a threefold increase in the rate of bacterial incorporation of [^3H]TdR into DNA from morning to afternoon and suggested that the response was related to changes in temperature and water chemistry. Similarly, our epilithic production rates may represent maxima because they were taken in the summer during peak daytime temperatures. However, we must stress that these rates may only be maximal estimates for the epilithon on the upper surfaces of rocks in riffles. Lock et al. (1984) reported substantial epilithic communities on lower rock surfaces in their streams. If this was true in our streams, then our rates may be underestimates of total epilithic production in the riffles.

Epilithic bacterial production (Fig. 4) and doubling times were related to temperature, but not to AFDM and water column phosphorus. Activities of lotic bacterial biofilms have been observed to be coupled to temperature (Bott 1975; Kaplan and Bott 1989) and to epilithic dry matter and AFDM (Sinsabaugh and Linkins 1988; Sorensen et al. 1988). The overestimates of AFDM at site LC may have obscured a relationship between production and AFDM (see Results for explanation). However, production was still not significantly related ($P > 0.05$) to AFDM when the data of site LC were removed from the regression.

Our understanding of the structure and function of the epilithic bacterial community in streams is growing (e.g. see Lock et al. 1984; Hamilton 1987). However, to our knowledge, only one other study (Stock and Ward 1989) has examined the production of natural epilithic bacterial communities. Their results ($1.7\text{--}4.1 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$), calculated from a forested stream, are similar to our rates in forested streams (Tables 5 and 6).

Comparison of Habitat Productivities

We can now make some provisional comparisons among rates of bacterial production in the various habitats of the forested and open streams, in terms of the average rate (in the common units milligrams carbon per square metre per hour). In addition, rates of bacterial production can be compared with concurrent data on primary production in the same streams (Rosenfeld and Roff 1991). Rates in the water column have been converted to a square metre basis by allowing for an average water depth of 10 cm above riffles and 30 cm in pools; such depths are characteristic of these habitats in our streams. Hourly rates instead of daily rates of bacterial production are reported because the diel variations in productivity (e.g. see Moriarty and Pollard 1982) are unknown in these habitats.

Our data indicate that the water column has the lowest rates of bacterial production of the three habitats. Bacterial production in the water column over riffles ($0.04 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) and in pools ($0.11 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) in forested streams is negligible compared with that in the other habitat types. In the water column of open streams, bacterial production was 100-fold higher ($4 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ over riffles, $12 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ in pools).

Undoubtedly, bacterial production in the water column in pools of open streams during base flow and high summer temperatures may become more important. Primary production within the water column is also low in these streams, less than $0.5 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$. However, in the summer, large algal mats can develop in local pools at times of extremely low discharge.

In forested streams, the sediment habitat has the greatest rate of bacterial production ($70.3 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$), with the epilithon contributing only $2.9 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$. In contrast, net primary production was $4.4 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ on sediments and $33 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ in the epilithon of forested streams.

Pool sediments of open streams supported the highest rates of bacterial production ($93 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$). These rates were higher than net primary production in the same sediments ($58 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$). In open streams, the rate of epilithic bacterial production ($28 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) is 14-fold less than periphytic production ($390 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$). This difference between bacterial and periphytic production would be less if we had integrated bacterial production throughout the depth of the riffle (e.g. to lower rock surfaces) and into the underlying sediment.

Clearly, rates of bacterial production are highest in sediment habitats, and they are quite similar in forested and open streams. Whether these rates on sediments are underestimated or not (see above), bacterial production is an important source of labile particulate carbon in our headwater streams. Given approximately equal lengths of riffle and pool reaches (a reasonable generalisation for these streams), bacterial production certainly predominates over primary production at forested reaches and contributes importantly (or even predominates in pools) at open reaches. In addition, bacterial production is probably even more significant if expressed on a daily basis because algal production ceases at night, while bacterial production continues, albeit at some lower rate.

Our results support the general conclusion (determined from the interpretation of *P/R* ratios) that heterotrophic processes predominate in forested streams. However, for our open streams, more complete measurements of bacterial production are required before such a conclusion can be drawn. For example, what is the bacterial production in the underlying substrata of riffles, and what are the daily cycles of production in the sediment, epilithic, and water column habitats? In addition, the production of other microheterotrophs in the system (e.g. fungi and protozoa) is unknown. Including the production of these microorganisms with bacterial production may show that open streams are more heterotrophic than we have traditionally believed.

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References

- ALONGI, D. M. 1988. Bacterial productivity and microbial biomass in tropical mangrove sediments. *Microb. Ecol.* 15: 59–79.
- APHA (AMERICAN PUBLIC HEALTH ASSOCIATION). 1980. Standard methods for the examination of water and wastewater. 14th ed. American Public Health Association, Washington, DC.
- ASHENDEN, J. E. 1986. Available energy, energy inventories and relationships between energy and secondary production in southern Ontario streams. M.Sc. thesis, University of Guelph, Guelph, Ont. 109 p.
- AUSTIN, H. K., AND S. G. FINDLAY. 1989. Benthic bacterial biomass and production in the Hudson River Estuary. *Microb. Ecol.* 18: 105–106.
- BARTON, D. R., W. D. TAYLOR, AND R. M. BIETTE. 1985. Dimensions of riparian buffer strips required to maintain trout habitat in southern Ontario streams. *M. Am. J. Fish. Manage.* 5: 364–378.
- BELL, R. T. 1986. Further verification of the isotope dilution approach for estimating the degree of participation of [^3H]-thymidine in DNA synthesis in studies of aquatic bacterial production. *Appl. Environ. Microbiol.* 52: 1212–1214.
- BELL, R., G. AHLGREN, AND I. AHLGREN. 1983. Estimating bacterio-plankton production by measuring [^3H]-thymidine incorporation in a eutrophic Swedish lake. *Appl. Environ. Microbiol.* 45: 1709–1721.
- BELL, R. T., AND I. AHLGREN. 1987. Thymidine incorporation and microbial respiration in the surface sediment of a hypereutrophic lake. *Limnol. Oceanogr.* 32: 476–482.
- BENKE, A. C., C. A. HALL, C. P. HAWKINS, R. H. LOWE-McCONNELL, J. A. STANFORD, K. SUBERKROPP, AND J. V. WARD. 1988. Bioenergetic considerations in the analysis of stream ecosystems. *J. N. Am. Benthol. Soc.* 7: 480–502.
- BOTT, T. L. 1975. Bacterial growth rates and temperature optima in a stream with a fluctuating thermal regime. *Limnol. Oceanogr.* 20: 191–197.
- BOTT, T. L., J. T. BROCK, C. S. DUNN, R. J. NAIMAN, R. W. OVINK, AND R. C. PETERSEN. 1985. Benthic community metabolism in four temperate stream systems: an interbiome comparison and evaluation of the river continuum concept. *Hydrobiologia* 123: 3–45.
- BOTT, T. L., AND L. A. KAPLAN. 1985. Bacterial biomass, metabolic state, and activity in stream sediments: relation to environmental variables and multiple assay comparisons. *Appl. Environ. Microbiol.* 50: 508–522.
- BOWLBY, J. N., AND J. C. ROFF. 1986. Trout biomass and habitat relationships in southern Ontario streams. *Trans. Am. Fish. Soc.* 115: 503–514.
- COLE, J. J., S. FINDLAY, AND M. L. PACE. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar. Ecol. Prog. Ser.* 43: 1–10.
- CUMMINS, K. W. 1974. Structure and function of stream ecosystems. *Bio-Science* 24: 631–641.
- DOBBS, F. C., J. B. GUCKERT, AND K. R. CARMANN. 1989. Comparison of three techniques for administering radiolabeled substrates to sediments for trophic studies: incorporation by microbes. *Microb. Ecol.* 17: 237–250.
- EDWARDS, R. T. 1987. Sestonic bacteria as a food source for filtering invertebrates in two southeastern blackwater rivers. *Limnol. Oceanogr.* 32: 221–234.
- EDWARDS, R. T., AND J. L. MEYER. 1986. Production and turnover of planktonic bacteria in two southeastern blackwater rivers. *Appl. Environ. Microbiol.* 52: 1317–1323.
1987. Bacteria as a food source for black fly larvae in a blackwater river. *J. N. Am. Benthol. Soc.* 6: 241–250.
- FINDLAY, S., J. L. MEYER, AND R. T. EDWARDS. 1984. Measuring bacterial production via rate of incorporation of [^3H]-thymidine into DNA. *J. Microb. Methods* 2: 57–72.
- FINDLAY, S., J. L. MEYER, AND R. RISLEY. 1986. Benthic bacterial biomass and production in two blackwater rivers. *Can. J. Fish. Aquat. Sci.* 43: 1271–1276.
- FISHER, S. G., AND G. E. LIKENS. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. *Ecol. Monogr.* 43: 421–439.
- GOULDER, R. 1988. Epilithic bacteria in an acid and a calcareous headstream. *Freshwater Biol.* 19: 405–416.
- HAACK, T. K., AND G. A. McFETERS. 1982a. Microbial dynamics of an epilithic mat community in a high alpine stream. *Appl. Environ. Microbiol.* 43: 702–707.
- 1982b. Nutritional relationships among microorganisms in an epilithic biofilm community. *Microb. Ecol.* 8: 115–126.
- HAMILTON, W. A. 1987. Biofilms: microbial interactions and metabolic activities, p. 361–385. *In* M. Fletcher, T. R. Gray, and J. G. Jones [ed.] *Ecology of microbial communities*. Cambridge University Press, Cambridge.
- HOLLIBAUGH, J. T. 1988. Limitations of the [^3H]-thymidine method for estimating bacterial productivity due to thymidine metabolism. *Mar. Ecol. Prog. Ser.* 43: 19–30.
- HUDSON, J. H., J. C. ROFF, AND B. K. BURNISON. 1990. Measuring epilithic bacterial production in streams. *Can. J. Fish. Aquat. Sci.* 47: 1813–1820.
- KAPLAN, L. A., AND T. L. BOTT. 1982. Diel fluctuations of DOC generated by algae in a piedmont stream. *Limnol. Oceanogr.* 27: 1091–1100.
1989. Diel fluctuations in bacterial activity on streambed substrata during vernal algal blooms: effects of temperature, water chemistry, and habitat. *Limnol. Oceanogr.* 34: 718–733.
- LOCK, M. A., R. R. WALLACE, J. W. COSTERTON, R. M. VENTULLO AND S. F. CHARLTON. 1984. River epilithon: toward a structural-functional model. *Oikos* 42: 10–22.

- MATSON, E. A., AND R. L. KLOTZ. 1983. Organic carbon supply and demand in the Sketucket river of eastern Connecticut, p. 247–272. *In* T. Fontaine and S. Bartell [ed.] Dynamics of lotic ecosystems. Ann Arbor Science Publishers, Ann Arbor, MI. 494 p.
- MEYER, J. L. 1988. Benthic bacterial biomass and production in a blackwater river. *Verh. Int. Ver. Limnol.* 23: 1832–1838.
1989. Can *P/R* ratio be used to assess the food base of stream ecosystems? A comment on Rosenfeld and Mackay (1987). *Oikos* 54: 119–121.
- MINSHALL, G. W. 1978. Autotrophy in stream ecosystems. *BioScience* 28: 767–771.
- MINSHALL, G. W., K. W. CUMMINS, R. C. PETERSEN, C. E. CUSHING, D. A. BRUNS, J. R. SEDELL, AND R. L. VANNOTE. 1985. Developments in stream ecosystem theory. *Can. J. Fish. Aquat. Sci.* 42: 1045–1055.
- MORIARTY, D. J. 1986a. Measurement of bacterial growth rates in aquatic systems using rates of nucleic acid synthesis. *Adv. Microbiol. Ecol.* 9: 245–292.
- 1986b. Bacterial productivity in ponds used for culture of panaeid prawns. *Microb. Ecol.* 12: 259–269.
- MORIARTY, D. J., AND P. C. POLLARD. 1981. DNA synthesis as a measure of bacterial productivity in seagrass sediments. *Mar. Ecol. Prog. Ser.* 5: 151–156.
1982. Diel variation of bacterial productivity in seagrass (*Zostera capricorni*) beds measured by rate of thymidine incorporation into DNA. *Mar. Biol.* 72: 165–173.
- PALUMBO, A. V., M. A. BOGLE, R. R. TURNER, J. W. ELWOOD, AND P. J. MULHOLLAND. 1987. Bacterial communities in acidic and circumneutral streams. *Appl. Environ. Microbiol.* 53: 337–344.
- POLLARD, P. C., AND D. J. W. MORIARTY. 1984. Validity of the tritiated thymidine method for estimating bacterial growth rates: measurement of isotope dilution during DNA synthesis. *Appl. Environ. Microbiol.* 48: 1076–1083.
- RHEINHEIMER, G. 1985. *Aquatic microbiology*. John Wiley & Sons, Toronto, Ont. 257 p.
- RIEMANN, B., AND M. SONDERGAARD. 1984. Measurements of diel rates of bacterial secondary production in aquatic environments. *Appl. Environ. Microbiol.* 47: 632–638.
- ROBERTS, R. D. 1988. Heterotrophic bacterial activity and primary production in a hypertrophic African lake. *Hydrobiologia* 162: 97–107.
- ROSENFELD, J. S., AND R. J. MACKAY. 1987. Assessing the food base of stream ecosystems: alternatives to the *P/R* ratio. *Oikos* 50: 141–147.
- ROSENFELD, J. S., AND J. C. ROFF. 1991. Primary production and potential availability of autochthonous carbon in southern Ontario streams. *Hydrobiologia* 224: 99–109.
- RUBLEE, P. 1982. Seasonal distribution of bacteria in salt marsh sediments in North Carolina. *Estuarine Coastal Shelf Sci.* 15: 67–74.
- SERVAIS, P. 1989. Bacterioplanktonic biomass and production in the river Meuse (Belgium). *Hydrobiologia* 174: 99–110.
- SINSABAUGH, R. L., AND A. E. LINKINS. 1988. Exoenzyme activity associated with lotic epilithon. *Freshwater Biol.* 20: 249–261.
- SONDERGAARD, M., AND L. M. JENSEN. 1986. Phytoplankton, p. 27–126. *In* B. Riemann and M. Sondergaard [ed.] Carbon dynamics in eutrophic, temperate lakes. Elsevier, New York, NY. 284 p.
- SORENSEN, J., T. JORGENSEN, AND S. BRANDT. 1988. Denitrification in stream epilithon: seasonal variation in Gelbaek and Rabis Baek, Denmark. *FEMS Microb. Ecol.* 53: 345–354.
- STOCK, M. S., AND A. K. WARD. 1989. Establishment of a bedrock epilithic community in a small stream: microbial (algal and bacterial) metabolism and physical structure. *Can. J. Fish. Aquat. Sci.* 46: 1874–1883.
- TAYLOR, B. R., AND J. C. ROFF. 1982. Evaluation of ecological maturity in three headwater streams. *Arch. Hydrobiol.* 94: 99–125.
- VANNOTE, R. L., G. W. MINSHALL, K. W. CUMMINS, J. R. SEDELL, AND C. E. CUSHING. 1980. The river continuum concept. *Can. J. Fish. Aquat. Sci.* 37: 130–137.
- WATSON, S. W., T. J. NOVITSKY, H. L. QUINBY, AND F. W. VALOIS. 1977. Determination of bacterial number and biomass in the marine environment. *Appl. Environ. Microbiol.* 33: 940–946.
- WICKS, R. J., AND R. D. ROBERTS. 1987. The extraction and purification of DNA labelled with [methyl-³H]thymidine in aquatic bacterial production studies. *J. Plankton Res.* 9: 1159–1166.

Patterns in Coastal Migration and Stock Structure of Capelin (*Mallotus villosus*)

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Nakashima, B. S. 1992. Patterns in coastal migration and stock structure of capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. 49: 2423–2429.

Approximately 57 500 mature capelin (*Mallotus villosus*) were tagged with external tags from 1983 to 1988 along the southeast and east coasts of Newfoundland to determine inshore migration patterns and to reexamine the current belief that two separate capelin stocks spawn on east coast beaches. Capelin released in a particular bay were recaptured from the same bay or locations further north. Upstream migration using the Labrador Current was hypothesized as a directional clue to the prespawning migration. Capelin caught in one stock area were recaptured in another area, indicating that mature capelin from the Northeast Newfoundland – Labrador and Northern Grand Bank – Avalon stocks mix and migrate north together. The distribution and mixture of prespawning fish inshore as described by tag returns have implications on how capelin resources should be surveyed, assessed, and managed.

Environ 57 500 capelans (*Mallotus villosus*) adultes ont été marqués avec des étiquettes externes entre 1983 et 1988 le long des côtes sud-est et est de Terre-Neuve afin de déterminer les profils de migration près des côtes et de réexaminer l'hypothèse actuelle selon laquelle deux stocks de capelans distincts fraient sur les plages de la côte est. Les capelans libérés dans une baie donnée ont été recapturés dans la même baie ou à des endroits situés plus au nord. On a émis comme hypothèse que la migration vers l'amont utilisant le courant du Labrador constituait un indice de la direction de la migration préreproductrice. Les capelans capturés dans la région d'un stock donné ont été recapturés dans une autre région, ce qui indique que les capelans adultes des stocks de la région nord-est de Terre-Neuve – Labrador et de la région Grand Banc septentrional – Avalon se mélangent et migrent ensemble vers le nord. La distribution et le mélange de poissons s'effectuant avant la fraie près des côtes et que l'on peut déduire des études de marquage a des conséquences sur la manière dont les ressources de capelans devraient être étudiées, évaluées et gérées.

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Assessment and management of a commercially important species such as capelin (*Mallotus villosus*) are closely linked to our understanding of migration patterns and the degree of separation among stocks. Capelin are distributed in the Northern Hemisphere in the Atlantic, Pacific, and Arctic oceans and occur from Hudson Bay to the southern Gulf of St. Lawrence in the northwest Atlantic Ocean. Each spring, mature capelin in the northwest Atlantic Ocean migrate from offshore feeding areas to spawn on beaches along the coastlines of Newfoundland and Labrador (Templeman 1948; Campbell and Winters 1973) or offshore on the Southeast Shoal (Carscadden et al. 1989). The nature of the migration, especially within coastal areas, remains largely unknown but one hypothesis is that once fish are near the coast or within the bay proper, they tend to spawn on nearby beaches.

Evidence to support the current stock structure used to assess and manage capelin resources on Newfoundland's east coast is tenuous and circumstantial. Campbell and Winters (1973) used seasonal distribution patterns in research vessel surveys to propose the hypothesis that capelin in the northwest Atlantic were composed of three inshore spawning stocks (Northeast Newfoundland – Labrador stock in NAFO SA2 + Div. 3K, Northern Grand Bank – Avalon stock in NAFO Div. 3L, St. Pierre Bank stock in NAFO Div. 3Ps) and one offshore spawning stock (the Southeast Shoal in NAFO Div. 3NO). Recent attempts to discriminate between fish from the Northeast Newfoundland – Labrador and Northern Grand Bank – Avalon

stocks have largely been unsuccessful (Sharp et al. 1978; Carscadden and Misra 1980; Palsson 1986). Misra and Carscadden (1984), in a reanalysis of their original meristic data published in 1980, were only able to differentiate between fish of these two stocks using paired comparisons. This raises the question whether the accepted stocks are biologically different or just statistically different.

A tagging programme within the bays of the southeast and east coasts of Newfoundland was designed to elucidate the extent to which capelin schools spawn in the same bays where they were found prior to spawning. I also considered the current stock designations used to set quotas which assume that mature capelin spawning inshore on beaches between Cape St. Mary's and Cape Freels (Fig. 1) constitute the Northern Grand Bank – Avalon stock and those spawning north of Cape Freels form the Northeast Newfoundland – Labrador stock.

Materials and Methods

External tags were chosen as the most visible means to obtain reliable information on location and date of recapture. Initially in 1983, I experimented with two external tag types, a short anchor (FLOY FD68BC, 20-mm yellow tubing) and a streamer (FLOY FTSL-73, 95-mm yellow ribbon). Each tag has an abbreviated return address and a sequential alphanumeric code to identify individual fish. During the 1983 experiments, we were able to apply streamer tags at a faster rate than short anchor

TABLE 1. Summary of location, date when commercial fishing began, date of release, and number of capelin tagged and released.

Year	Bay	Date fishing began	Location	Release date	No. of tags released	
					Males	Females
1983	Conception	June 14	Upper Island Cove	May 29	1275	350
			Salmon Cove	May 31	525	1025
			Harbour Grace Islands	June 2	600	825
			Salmon Cove	June 21	1950	1350
			Salmon Cove	June 23	400	2650
			Bacon Cove	June 27	1125	2075
			Harbour Grace Islands	June 28	1800	1775
			Total		7675	10050
1984	Conception	June 11	Port de Grave	May 23	800	900
			Broad Cove	May 24	600	200
			Salmon Cove	May 25	700	300
			Upper Island Cove	May 26	600	400
			Bacon Cove	May 26	300	300
			Western Bay	May 29	1100	1300
	Trinity	June 13	East Random Head	June 6	600	600
			Horse Chops	June 14	900	300
			Spaniards Cove	June 21	1800	300
			Total		7400	4600
1985	Conception	June 27	Lower Island Cove	May 27	700	800
	Trinity	June 27	Southwest Arm	June 1	300	300
			Southwest Arm	June 18	400	500
			Bonaventure Head	June 25	700	400
			Melrose	June 26	400	400
			Horse Chops	June 26	500	400
			Trinity	June 27	300	600
	Bonavista	June 28	Greenspond	June 29	500	500
			Bessie's Island	July 1	400	600
			Bessie's Island	July 2	700	700
			Sandy Cove	July 3	400	300
			Total		5300	5500
1986	Trinity	June 5	Trinity	May 24	400	700
	Conception	June 13	Bonaventure Head	May 24	600	600
			Broad Cove	June 7	300	600
			Total		1300	1900
1987	St. Mary's	June 18	Colinet Island	May 29	600	600
			Little Salmonier Point	May 30	600	600
			Harricot Point	May 31	500	500
			Colinet Island	May 31	400	400
			Colinet Island	June 1	300	300
	Trinity	June 19	Trinity	June 10	600	600
			Smith Sound	June 11	500	500
			Sandy Cove	June 13	300	300
	Bonavista	June 19	Greenspond	June 14	400	400
			Fair Island	June 19	250	600
			Bloody Bay Reach	June 20	450	400
			Bessie's Island	June 20	400	400
			Long Island	June 21	50	500
			Cape Fogo	June 17	700	700
	Notre Dame	June 19	Total		6050	6800
1988	St. Mary's	June 15	Colinet Island	June 13	449	499

tags. Also, short anchor tags caused more damage, especially to females which are smaller than males (Templeman 1948). Therefore, since 1984 only streamer tags have been used.

Extensive searching was conducted daily to tag capelin within the bays prior to spawning in May and June. One (1987 and

1988) or two (1983–86) purse seiners were deployed to catch capelin. Release sites are given in Table 1. When a school was caught in a purse seine, small numbers of capelin were carefully dipped aboard and held briefly on deck in plastic containers (130 L) filled with seawater. Only mature fish (i.e. those that

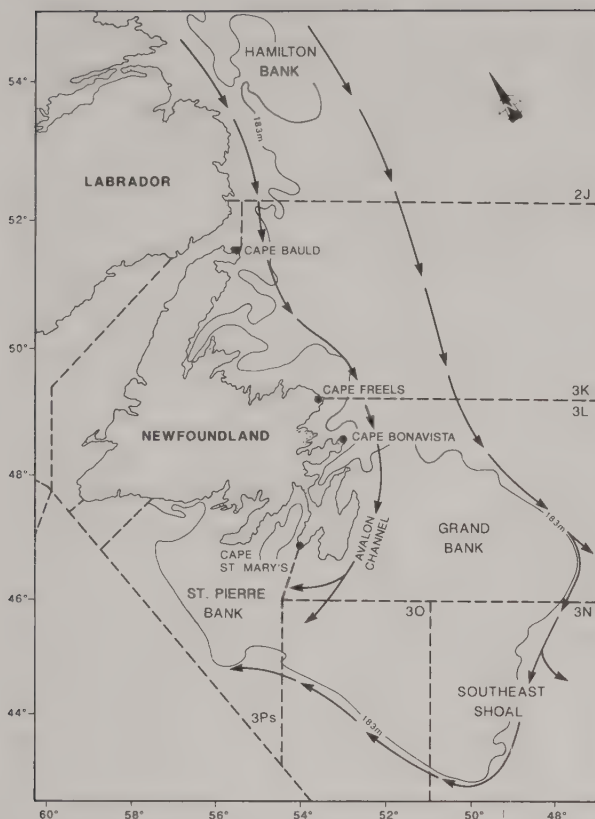


FIG. 1. NAFO divisions, place names, and location of the Labrador Current on the east and southeast coasts of Newfoundland.

would spawn in June or July of that year) in good condition were tagged. Tagging continued until fish appeared sluggish or sufficient numbers had been tagged. To minimize initial mortalities, we tagged fish in as short a time as possible (generally 1 h after capture), assuming that mortality increased with handling and holding time as shown for Atlantic herring (*Clupea harengus*) (Nakashima and Winters 1984). No experiments were conducted to estimate handling and tagging mortality.

Recaptures of tagged fish were expected from fishermen, fish plant workers, and the general public. Because capelin caught in the commercial fishery were handled to test for quality, to separate males from females, and to pack females into boxes on production lines the potential for detecting external yellow tags was high. Also, capelin spawn on exposed intertidal areas of gravel beaches, thus making external tags visible. Posters were displayed in prominent locations advertising the tagging programme and offering a \$5.00 reward. Repeated announcements were made during the fishing season on a province-wide radio programme which focuses on fishery-related issues.

Recapture information was carefully scrutinized. The nature of the fishery was such that exact time and location of capture may have been difficult to determine, especially from fish plant workers. Factors that could have led to poor information were trucking of the catch to different fish plants, inability by plant workers on the production line to know where the catch was actually taken, and length of time taken from when the tag was found and when it was reported. Tag recovery information was verified with telephone calls or a mailed questionnaire. Relia-



FIG. 2. Locations where tagged capelin were released (solid symbols) during the fishery in Conception Bay on June 21–28, 1983 (squares), and in Trinity Bay on June 21, 1984 (circles), and corresponding recapture sites (open symbols). The fraction has standardized returns in the numerator and actual returns in the denominator.

bility codes for time and location of capture were assigned to each tag (1 = highly reliable, 2 = some doubt but acceptable, 3 = unreliable). All returns were considered when estimating total recapture rates, but only those assigned a reliability code of 1 were used to address questions regarding migration patterns and stock mixtures.

To examine migration patterns, all tag types and years of release were combined according to the bay where tagged fish were released. The actual number of recaptures and the number of recaptures adjusted for commercial landings were estimated for each bay. Fishing effort varied among bays and among years sufficiently to influence the number of recaptures. Because effort statistics were unavailable for every area for all years, reported landings by bay provided by the Statistics Branch of the Department of Fisheries and Oceans were assumed to represent effort, and recaptures were standardized as number of recaptures/10 000 t landed.

Results

Standardized recaptures show that fish tagged during the fishery were generally caught in the bay where they were released (Fig. 2) compared with capelin tagged in the same areas before the fishery began (Fig. 3 and 4) which showed considerable northward movement away from the release site. For these comparisons, I assumed that fishing began in each

TABLE 2. Annual release and recapture data for streamer (S) and anchor (A) tag experiments during 1983–88.

Year released	No. of tags released	Tag type	Sex	Year recaptured		Percentage recaptured
				Same	Second	
1983	3450	A	M	62	1	1.8
	3500	S	M	105		3.0
	1075	A	F	13		1.2
	9700	S	F	262	5	2.8
1984	9900	S	M	265	9	2.8
	4600	S	F	107	1	2.4
1985	5300	S	M	102		1.9
	5500	S	F	62		1.1
1986	1300	S	M	23	1	1.8
	1900	S	F	21	1	1.2
1987	6050	S	M	41	2	0.7
	6800	S	F	45		0.7
1988	449	S	M	3		0.7
	499	S	F	3		0.6

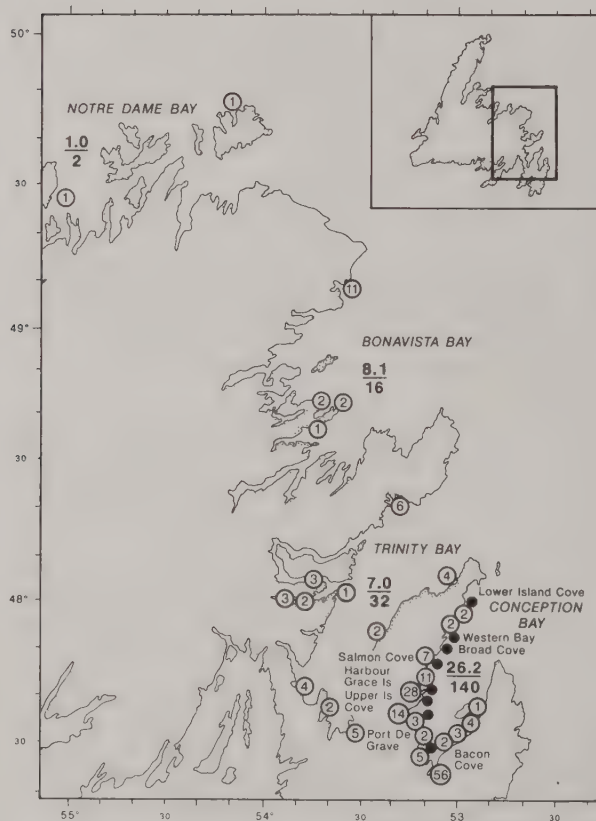


FIG. 3. Locations where tagged capelin were released in Conception Bay from 1983 to 1986 (solid circles) and recapture sites (open circles). The fraction has standardized returns in the numerator and actual returns in the denominator.

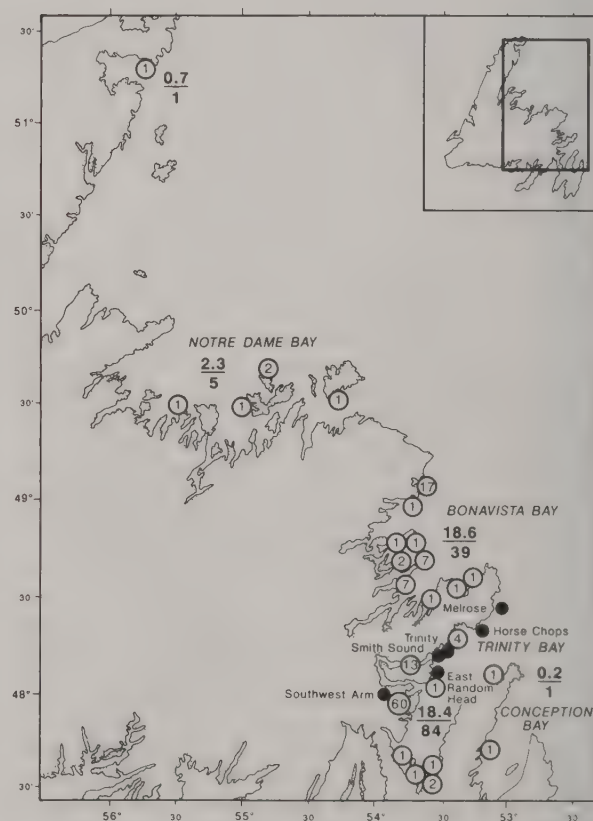


FIG. 4. Locations where tagged capelin were released in Trinity Bay from 1984 to 1987 (solid circles) and recapture sites (open circles). The fraction has standardized returns in the numerator and actual returns in the denominator.

bay (Table 2) when the first 1000 t was landed and landings continued to increase thereafter. These comparisons suggest that information on fish movements from tagged fish released during the fishery was of limited use.

Out of 57 523 fish tagged from 1983 to 1988 in this study, 20 tagged fish were recovered the following year (Table 2), indicating that they were spawning for a second time. Of these 20 fish, eight were originally tagged and released in Conception

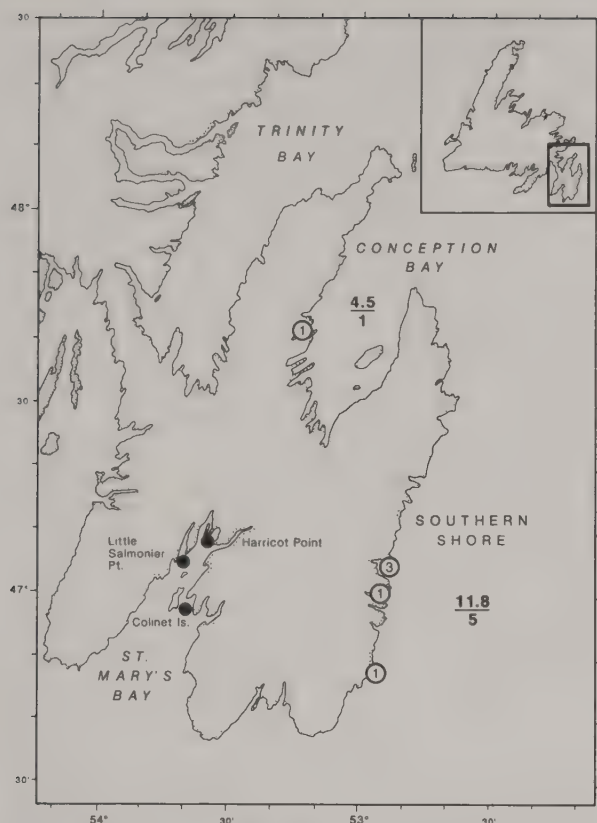


FIG. 5. Locations where tagged capelin were released in St. Mary's Bay from 1987 and 1988 (solid circles) and recapture sites (open circles). The fraction has standardized returns in the numerator and actual returns in the denominator.

Bay and 12 in Trinity Bay. From the Conception Bay releases, six were returned from Conception Bay, one from Trinity Bay, and one from the Southern Shore. From the Trinity Bay taggings, five were returned from Trinity Bay, three from Conception Bay, three from Bonavista Bay, and for one return the recapture site was unknown. While the numbers are small compared with the numbers released, they do demonstrate that some proportion of each year's spawners consists of repeat spawners. In May 1966, Winters (1971) found repeat spawning females based on the previous year's spawning zones on otoliths.

Mature capelin tagged along coastal areas of southeastern and eastern Newfoundland in May and June prior to spawning remain in the same area to spawn later or migrate to spawn on beaches further north and west (Fig. 3–7). During two years of tagging in St. Mary's Bay, five capelin were recaptured further north on the Southern Shore and one in Conception Bay (Fig. 5). No marked capelin released in St. Mary's Bay were recaptured in St. Mary's Bay. Fish tagged in Conception Bay were found in that bay itself or further north in Trinity, Bonavista, and Notre Dame bays (Fig. 2 and 3). Capelin tagged in Trinity Bay were recaptured from that bay and from bays further north such as Bonavista, Notre Dame, and White bays (Fig. 2 and 4), except for one tag from Conception Bay. Fish tagged in Bonavista Bay were caught in Bonavista and Notre Dame bays (Fig. 6). Capelin tagged near Fogo Island, Notre Dame Bay, were recaptured in Notre Dame Bay, west of the release point and upstream of the Labrador Current (Fig. 7).



FIG. 6. Locations where tagged capelin were released in Bonavista Bay from 1985 and 1987 (solid circles) and recapture sites (open circles). The fraction has standardized returns in the numerator and actual returns in the denominator.

The only instances of recaptures downstream of the Labrador Current were five capelin recaptured in Conception Bay having been released in Trinity Bay (Fig. 2 and 4). Except for these five fish, all recaptures were returned from the bay of release or from locations north of the release point.

Tag return data show that capelin from two stock areas, the Northeast Newfoundland – Labrador and Northern Grand Bank – Avalon, intermingle during their inshore spawning migration as observed by tag returns north of Cape Freels in Div. 3K of fish captured and released in Div. 3L (Fig. 3, 4, and 6).

Discussion

The observed pattern along the east coast is consistent with contranant migration of mature adults returning to spawning beaches from which larvae dispersed (Harden Jones 1968). Larvae emerging from intertidal sediments in June and July are rapidly advected from embayments into the open bay in as little as 6–8 h (Taggart and Leggett 1987). Ichthyoplankton surveys in Trinity (E. Dalley, Northwest Atlantic Fisheries Centre, St. John's, Nfld., pers. comm.) and conception (J. T. Anderson, Northwest Atlantic Fisheries Centre, St. John's, Nfld., pers. comm.) bays in July and August show that larvae were displaced by the current flowing out of the southeastern side of these bays and onto the Grand Banks by late summer. Soviet prerecruit surveys in November and December found signifi-

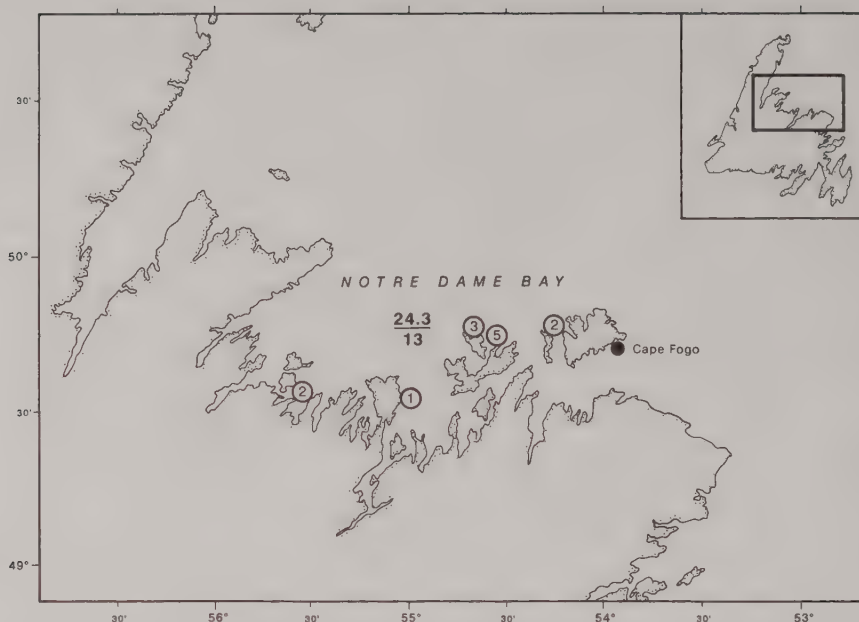


FIG. 7. Location where tagged capelin were released in Notre Dame Bay on June 17, 1987 (solid circles) and recapture sites (open circles). The fraction has standardized returns in the numerator and actual returns in the denominator.

cant concentrations of 0-group and 1-yr-old capelin between 43 and 48°N latitude on the Grand Banks (Bakanev and Oganin 1988; Bakanev et al. 1990). During annual Canadian acoustic surveys on the Grand Bank in NAFO Div. 3L in May, Miller and Carscadden (1989) observed that older, mature capelin occur in the southern part of the survey area and younger, smaller fish in the north. These separate survey results, taken together, suggest a denatant migration of larvae from spawning beaches onto the Grand Banks with a general southerly migration as fish become older. My tagging results indicate that there is a contranant migration in the spring of prespawning adults north to spawn on coastal beaches to complete the cycle.

The most likely mechanism employed by prespawning capelin is to migrate upstream against a detectable current. The Labrador Current (Fig. 1) flows southward along the Labrador coast and east coast of Newfoundland (Petrie and Anderson 1983). Near Cape Bonavista, the Labrador Current divides into an offshore and an inshore branch, with the inshore branch proceeding south near the coastline following the Avalon Channel (Petrie and Anderson 1983). Bailey (1958) observed a counterclockwise current in Trinity Bay in September. Yao (1986) showed that the alongshore component of the current was on the northwest side moving into Trinity Bay and moving out on the southeast side. The southward flow of the Labrador Current, especially the inshore branch and the counterclockwise current flow inside the long bays, may provide the directional clue capelin use to follow a northward countercurrent migration. When searching each bay for capelin to tag, schools were consistently located in areas where they could take advantage of the northwest side of the current flow into Trinity and possibly Conception bays, the inshore branch through the Avalon Channel outside St. Mary's Bay, and the Labrador Current itself flowing from the northern tip of the island southward onto the Grand Banks.

Other fish species such as herring, salmon, cod, eels, and plaice respond to water currents with rheotactic swimming movements (e.g. Harden Jones 1968; Arnold 1974, 1981; Westerberg 1979, 1984). Fish respond to water currents as slow as 1–5 cm/s (Harden Jones 1968) which encompasses the range of estimates for the Labrador Current off Labrador measured as 5.5 cm/s and as 4.3 cm/s for its inshore branch in the Avalon Channel (Greenberg and Petrie 1988). A countercurrent mechanism for capelin seems intuitively appealing, but some other clue such as water temperature or olfactory signals may eventually be discovered as the true mechanism.

The observed northward migration of spawning capelin along the coast in the spring agrees with anecdotal observations suggesting that the spring feeding migrations of cod are timed to and based on capelin migrations (Aggett et al. 1987). Templeman and Fleming (1962) reported that cod tagged on the southern Grand Bank migrated north early in the summer against the Labrador Current. More recently the distribution of tag returns during the 1990 inshore cod fishery in Newfoundland demonstrated that cod tagged and released in May 1990 on the northern Grand Bank migrated to inshore areas in the spring and then moved progressively northward through the summer along the east and northeast coasts (G. Rose, Northwest Atlantic Fisheries Centre, St. John's, Nfld., pers. comm.). The northward migration of capelin also supports the observed northward progression in capelin beach spawning times with latitude (Templeman 1948).

Two interpretations exist: (1) there are two distinct stocks (Northeast Newfoundland – Labrador and Northern Grand Bank – Avalon) at spawning which mix extensively prior to spawning or (2) capelin along the southeast and east coast of Newfoundland constitute one stock complex. Either interpretation has profound consequences for the way acoustic biomass surveys are done and fisheries management decisions are made.

In the first case which is the present basis for management decisions (Campbell and Winters 1973), tagging results demonstrate that capelin from the Northeast Newfoundland – Labrador stock migrate as far south as Conception Bay and mix with the Northern Grand Bank – Avalon stock before migrating north to spawn in Notre Dame and White bays. Acoustic surveys of these two stocks may not be covering their total area of distribution. The surveys assume that these stocks do not overlap and that they are entirely distributed in the survey area at the time. It is conceivable that some portion of the Northeast Newfoundland – Labrador stock may be south of the current fall acoustic survey from Hamilton Bank to Cape Freels (Fig. 1). In the second case, capelin from St. Mary's Bay to Cape Bauld constitute a single stock complex based on the general distribution of tag returns of tagged capelin released in St. Mary's, Conception, Trinity, and Bonavista bays (Fig. 3–6). This would explain the difficulty in differentiating capelin into two distinct stocks, one from the Northeast Newfoundland – Labrador area and the other from the Northern Grand Bank – Avalon area (Sharp et al. 1978; Carscadden and Misra 1980; Misra and Carscadden 1984; Palsson 1986). If there is a single capelin stock from Cape St. Mary's to Cape Bauld, it would require changes in timing and coverage of acoustic surveys and catch and quota assignments in management plans.

Current tag return data cannot conclusively establish which case is correct. However, they show that during the annual spawning migration along the coast, populations from separate spawning sites cooccur in the same area. Two types of fish, therefore, are caught in the bays prior to spawning: (1) maturing individuals waiting in the area for the right environmental conditions to initiate spawning and (2) individuals intercepted during their migration to spawning beaches further north. Migration routes that maturing capelin follow from offshore feeding areas to coastal Newfoundland each spring remain to be investigated.

Acknowledgments

The results would not have been realized without the capable assistance of Pelagic Section technicians, COSEP students, and the captains and their crews of the *Lone Flier*, *Silas T. Eastern Endeavor*, *Dean Brothers*, *Random Bell*, and *MARINUS* who participated in the tagging experiments. R. Harnum diligently scrutinized and coded the recapture information. An earlier draft benefited from reviews by G. H. Winters and J. B. Dempson. Comments by two anonymous reviewers helped to clarify and focus the manuscript. M. Hynes assisted in the preparation of the manuscript.

References

- AGGETT, D., H. S. GASKILL, D. FINLAYSON, S. MAY, C. CAMPBELL, AND J. BOBBITT. 1987. A study of factors influencing availability of cod in Conception Bay, Newfoundland, in 1985. *Can. Tech. Rep. Fish. Aquat. Sci.* 1562: 123 p.
- ARNOLD, G. P. 1974. Rheotropism in fishes. *Biol. Rev.* 49: 515–576.
1981. Movements of fish in relation to water currents, p. 55–79. *In* D. J. Aidley [ed.] *Animal migration*. Cambridge University Press, Cambridge.
- BAILEY, W. B. 1958. Trinity Bay, Newfoundland survey — September 1956. *Fish. Res. Board Can. MS Rep. Ser.* 10: 12 p. + 24 fig.
- BAKANEV, V. S., AND I. A. OGANIN. 1988. Ichthyoplankton investigations for the capelin 1987 year-class strength in NAFO Div. 3LNO. NAFO SCR Doc. 88/18: 11 p.
- BAKANEV, V. S., A. A. VASKOV, AND V. N. PETROV. 1990. Results of the Soviet acoustic survey on capelin stock in summer 1989 and 0-group capelin survey in autumn 1988 and 1989 in Div. 3LNO. NAFO SCR Doc. 90/7: 10 p.
- CAMPBELL, J. S., AND G. H. WINTERS. 1973. Some biological characteristics of capelin, *Mallotus villosus*, in the Newfoundland area. *ICNAF Redb.* 1973(III): 137–144.
- CARSCADDEN, J. E., K. T. FRANK, AND D. S. MILLER. 1989. Capelin (*Mallotus villosus*) spawning on the southeast shoal: influence of physical factors past and present. *Can. J. Fish. Aquat. Sci.* 46: 1743–1754.
- CARSCADDEN, J. E., AND R. K. MISRA. 1980. Multivariate analysis of meristic characters of capelin (*Mallotus villosus*) in the Northwest Atlantic. *Can. J. Fish. Aquat. Sci.* 37: 725–729.
- GREENBERG, D. A., AND B. D. PETRI. 1988. The mean barotropic circulation on the Newfoundland shelf and slope. *J. Geophys. Res.* 93: 15541–15550.
- HARDEN JONES, F. R. 1968. *Fish migrations*. Edward Arnold Ltd., London. 325 p.
- MILLER, D. S., AND J. E. CARSCADDEN. 1989. Biomass estimates from two hydroacoustic surveys for capelin (*Mallotus villosus*) in NAFO Divisions 3L and 3N and observations of the Soviet fishery for capelin in Division 3NO. NAFO SCR Doc. 89/52: 15 p.
- MISRA, R. K., AND J. E. CARSCADDEN. 1984. Stock discrimination of capelin (*Mallotus villosus*) in the Northwest Atlantic. *J. Northwest Atl. Fish. Sci.* 5: 199–205.
- NAKASHIMA, B. S., AND G. H. WINTERS. 1984. Selection of external tags for marking Atlantic herring (*Clupea harengus harengus*). *Can. J. Fish. Aquat. Sci.* 41: 1341–1348.
- PALSSON, J. 1986. Quantitative studies on the helminth fauna of capelin (*Mallotus villosus* Muller) in the northwest Atlantic for the purpose of stock discrimination. *Can. Tech. Rep. Fish. Aquat. Sci.* 1497: 21 p.
- PETRIE, B., AND C. ANDERSON. 1983. Circulation on the Newfoundland continental shelf. *Atmosphere-Ocean* 21: 207–226.
- SHARP, J. C., K. W. ABLE, W. C. LEGGETT, AND J. E. CARSCADDEN. 1978. Utility of meristic and morphometric characters for identification of capelin (*Mallotus villosus*) stocks in Canadian Atlantic waters. *J. Fish. Res. Board Can.* 35: 124–130.
- TAGGART, C. T., AND W. C. LEGGETT. 1987. Wind-forced hydrodynamics and their interaction with larval fish and plankton abundance: a time-series analysis of physical-biological data. *Can. J. Fish. Aquat. Sci.* 44: 438–451.
- TEMPLEMAN, W. 1948. The life history of the caplin (*Mallotus villosus*, O. F. Muller) in Newfoundland waters. *Nfld. Dep. Nat. Resour. Res. Bull.* 17: 1–151.
- TEMPLEMAN, W., AND A. FLEMING. 1962. Cod tagging in the Newfoundland area during 1947 and 1948. *J. Fish. Res. Board Can.* 19: 445–487.
- WESTERBERG, H. 1979. Counter-current orientation in the migration of the European eel. *Rapp. P.-V. Réun. Cons. Int. Explor. Mer* 174: 134–143.
1984. The orientation of fish and the vertical stratification at fine- and micro-structure scales, p. 179–203. *In* J. D. McCleave, G. P. Arnold, J. J. Dodson, and W. H. Neill [ed.] *Mechanisms of migration in fishes*. Plenum Press, New York, NY.
- WINTERS, G. H. 1971. Fecundity of the left and right ovaries of Grand Bank capelin (*Mallotus villosus*). *J. Fish. Res. Board Can.* 28: 1029–1033.
- YAO, T. 1986. The response of currents in Trinity Bay, Newfoundland, to local wind forcing. *Atmosphere-Ocean* 24: 235–252.

ANNOUNCEMENTS/AVIS

Third International Zebra Mussel Conference '93

February 23–26, 1993
Westin Harbour Castle
Toronto, Ontario, Canada

This four-day conference will highlight current research into the biology and impact of zebra mussels as well as the latest research on control options and systems developed to cope with the mussels. Abstracts and papers submitted will be collated for distribution. Selected full-length papers will be published by the Electric Power Research Institute (EPRI).

NOTE: This is the only major Zebra Mussel Conference to be held in North America in 1993. It combines the previous conferences sponsored by the Great Lakes Sea Grant Network, the Electric Power Research Institute, and various Canadian agencies.

Plenary Session for All Participants: February 23, 1993

- Overview of Exotic Species Introduction in North America
- Introduction to the Biology and Ecology of the Zebra Mussel
- Synthesis of Canadian and U.S. Zebra Mussel Programs
- Developments in the Regulatory Environment U.S. and Canada (i.e. Ballast Water Control, Mitigation Option, Limitations of Spread, etc.)
- Economic Impact
- European Overview

Current Sessions: February 24, 25, and 26, 1993

Biology

- Biology of the Zebra Mussel
- Population Dynamics
- Ecological Impacts
- Other Exotic Species
- Monitoring and Assessment

Control

- Mitigation Strategies — Case Studies
- Chemical Control Options
- Biofouling and Corrosion
- Biological Control Options
- Physical Control Options

For further information, Contact Chris Brousseau (416) 832-7113 or Renata Claudi (416) 506-6874. Zebra Mussel Coordinating Office, P.O. Box 5000, Maple, Ont. L6A 1S9, Canada.

Troisième conférence internationale sur la moule zébrée 1993

du 23 au 26 février 1993
Westin Harbour Castle
Toronto (Ontario), Canada

Cette conférence d'une durée de quatre jours sera axée sur les recherches actuellement réalisées dans les domaines de la biologie et des incidences de la moule zébrée ainsi que sur les études les plus récentes des moyens et systèmes de lutte contre cette moule. Les résumés et communications soumis seront diffusés. Certaines communications seront publiées dans leur entier par le Electric Power Research Institute (EPRI).

Note : Cette conférence est la seule conférence d'envergure sur la moule zébrée qui sera tenue en Amérique du Nord en 1993. Elle regroupe les conférences antérieurement parrainées par le Great Lakes Sea Grant Network, le Electric Power Research Institute et divers autres organismes canadiens.

La séance plénière du 23 février 1993 s'adresse à tous les participants et portera sur :

- Aperçu de l'introduction d'espèces exotiques en Amérique du Nord
- Introduction à la biologie et à l'écologie de la moule zébrée
- Synthèse des programmes canadiens et américains sur la moule zébrée
- Éléments nouveaux de la réglementation américaine et canadienne (contrôle des eaux de ballast, réduction des incidences, limitation de la dispersion, etc.)
- Incidences économiques
- Aperçu de la situation européenne

Séances des 24, 25 et 26 février 1993

Biologie

- Biologie de la moule zébrée
- Dynamique de population
- Incidences écologiques
- Autres espèces exotiques
- Contrôle et évaluation

Lutte

- Stratégies d'atténuation des incidences — études de cas
- Options de lutte chimique
- Biosalissures et corrosion
- Options de lutte biologique
- Options de lutte physique

Il est possible d'obtenir des précisions supplémentaires en s'adressant à Chris Brousseau (416) 832-7113 ou à Renata Claudi (416) 506-6874. Zebra Mussel Coordinating Office, C.P. 5000, Maple (Ontario) L6A 1S9, Canada.

Notice

The Wildlife Toxicology Fund supports high-calibre research in the field of wildlife toxicology

Applications are being received for practical and applied wildlife toxicology research which focuses on establishing the significance of impacts of toxic substances on wildlife and its habitat. Priority areas include research on

- pesticides and pest control practices
- the impact of substances from controllable or site-specific sources in a manner which could lead to specific or generic remedial measures
- identifying **indirect impacts** of toxic substances on wildlife and its habitat

Please read the "Guidelines for Applicants" before applying; copies can be obtained through the Program Manager, Wildlife Toxicology Fund, c/o World Wildlife Fund, 90 Eglinton Avenue East, Suite 504, Toronto, Ont. M4P 2Z7, Canada. Telephone: (416) 489-8800; Fax: (416) 489-3611. Deadline for application is **April 15, 1993**.

The Wildlife Toxicology Fund is a partnership of World Wildlife Fund Canada, Environment Canada (Canadian Wildlife Service), and the Natural Sciences and Engineering Research Council of Canada (NSERC).

Exxon Valdez Oil Spill Symposium

February 2-5, 1993
Egan Convention Center,
Anchorage, Alaska

Sponsors

Exxon Valdez Oil Spill Trustee Council, University of Alaska Sea Grant College Program, and American Fisheries Society, Alaska Chapter.

The Exxon Valdez Oil Spill Symposium is a forum dedicated to presentation of the results of the scientific studies conducted following the oil spill in Prince William Sound on March 24, 1989.

The first day will be devoted to overview presentations directed toward the general public. Everyone interested in the results of the oil spill research is encouraged to attend. There will be a social hour following the presentations so that attendees can meet and talk to the Exxon Valdez Oil Spill Trustee Council members and scientists who carried out the studies. Presentations will begin at 8:30 a.m. There is no charge for attending the first day of the Symposium.

The remaining three days (Technical Sessions) will be devoted to presentation of scientific papers. These presentations will focus on the results of the research conducted for up to four years following the oil spill by the Trustee Council agencies and other organizations. There will be a preregistration fee of \$95 for the three-day Technical Session.

A final agenda will be mailed to all registrants prior to the Symposium.

Announce

Le Fonds de la toxicologie pousse la recherche scientifique dans le domaine de la toxicologie faunique

Nous recevons présentement des demandes de subvention pour la recherche en toxicologie faunique appliquée dont le but est d'établir quels sont les impacts des substances toxiques sur la faune et les habitats fauniques. Les principales études doivent porter sur :

- les pesticides et les méthodes de contrôle des espèces nuisibles
- l'impact de substances provenant de sources qui peuvent être contrôlées ou de sources spécifiques; ces études devraient amener à des mesures de redressement spécifiques ou génériques
- l'identification des **impacts indirects** des substances toxiques sur la faune et les habitats fauniques

Veuillez lire les directives avant de remplir le formulaire de demande. Pour vous en procurer une copie, prenez contact avec le responsable du programme de toxicologie faunique, Fonds mondial pour la nature, 90 avenue Eglinton Est, bureau 504, Toronto (Ontario) M4P 2Z7, Canada. Téléphone : (416) 489-8800; télécopieur : (416) 489-3611. Le date limite est le **15 avril 1993**.

Le financement du programme de toxicologie faunique est rendu possible grâce à la collaboration du Fonds mondial pour la nature Canada, Environnement Canada (Service canadien de la faune) et le Conseil de recherches en sciences naturelles et en génie du Canada.

Congrès sur le déversement de l'Exxon Valdez

2 au 5 février
Egan Convention Center, Anchorage (Alaska)

Commanditaires

Conseil d'administration pour le déversement de l'Exxon Valdez, University of Alaska Sea Grant College Program et American Fisheries Society, Alaska Chapter.

Le Congrès sur le déversement de l'Exxon Valdez a pour objet de présenter les résultats des études scientifiques menées à la suite du déversement du 24 mars 1989 dans le golfe du Prince-William.

Au cours de la première journée, des présentations générales seront données à l'intention du grand public. Toutes les personnes intéressées par les résultats des études sont invitées. Les présentations seront suivies d'un volet social d'une heure au cours duquel les participants pourront discuter avec les membres du conseil d'administration pour le déversement de l'Exxon Valdez et les scientifiques qui ont mené les études. Les présentations commenceront à 8 h 30, et l'entrée est libre la première journée.

Les trois jours qui suivront sont réservés à la présentation de documents scientifiques (séances techniques). Ces présentations mettront l'accent sur les résultats des recherches qu'ont menées les comités du conseil d'administration et d'autres organismes. Les frais de pré-inscription pour ces trois journées sont de 95 \$.

Un programme définitif sera envoyé à tous les participants avant le congrès.

When and Where

The Symposium will be held February 2–5, 1992, at the William A. Egan Civic and Convention Center, 555 West 5th Avenue, Anchorage, Alaska.

Registration

If you plan to attend only the first day of the Symposium, there is no charge. If you plan to attend all of the Symposium and hear the presentations of research results, a preregistration fee of \$95.00 will be charged. At the door, registration fee will be \$110. A book of extended abstracts of the papers being presented will be available to all who register for the Technical Sessions. To register, return the attached registration form by **January 2, 1993**. Make checks or money orders payable to **The American Fisheries Society, Alaska Chapter**.

Proceedings

Proceedings of the *Exxon Valdez* Oil Spill Symposium will be published following the Symposium. Information on ordering the Proceedings will be available at the Symposium.

For further information, contact Brenda Baxter, Coordinator, University of Alaska Fairbanks Sea Grant College Program, Fairbanks, AK 99775-5040, USA. Telephone: (907) 474-7086; fax: (907) 474-6285; e-mail BITNET:FNBRM1@ALASKA,INTERNET:FNBRM1@ACAD3.ALASKA.EDU.

Date et lieu

Du 2 au 5 février 1993, William A. Egan Civic and Convention Center, 555 West 5th Avenue, Anchorage (Alaska).

Inscription

L'entrée est libre à la première journée du congrès. Les frais d'inscription pour les présentations des trois autres journées sont de 95 \$ à la pré-inscription et de 110 \$ à l'entrée. Des résumés approfondis des documents présentés seront distribués aux personnes qui s'inscrivent aux séances techniques. Pour vous inscrire, veuillez retourner la formule ci-jointe au plus tard **le 2 janvier 1993**. Chèques et mandats doivent être faits à l'ordre de **The American Fisheries Society, Alaska Chapter**.

Actes

Les actes du congrès seront publiés à la suite de celui-ci. Des bons de commande seront distribués au congrès.

Renseignements : Brenda Baxter, Coordonnatrice, University of Alaska Fairbanks Sea Grant College Program, Fairbanks (Alaska) 99775-5040, États-Unis. Téléphone : (907) 474-7086; télécopieur : (907) 474-6285; courrier électronique : BITNET: FNBRM1@ALASKA,INTERNET:FNBRM1@ACAD3.ALASKA.EDU.

Frank, K. T. Demographic consequences of age-specific dispersal in marine fish populations	2222-2231
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